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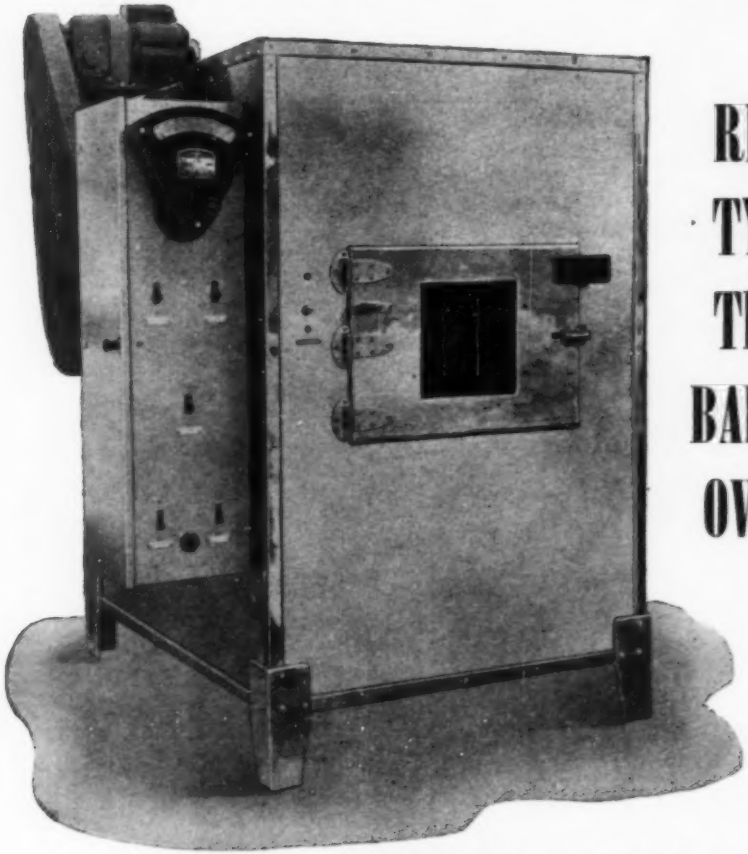

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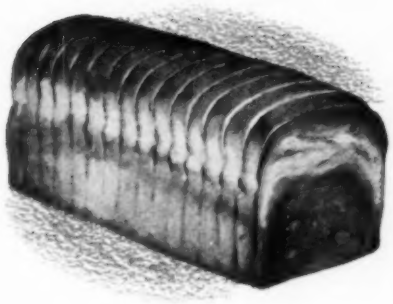

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CEREAL CHEMISTRY

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FRACTIONATION AND RECONSTITUTION PROCEDURES FOR CAKE FLOURS[1]

WILLIAM F. SOLLARS[2]

ABSTRACT

Bleached and unbleached cake flours were separated into water-solubles, gluten, tailings starch, and prime starch by an acetic acid fractionation procedure. Flour was first extracted with water to obtain the water-solubles which were stored as a liquid concentrate. The residue was then extracted with dilute acetic acid at pH 3.5, and the extract was neutralized to recover the gluten. The final two-layer residue was separated into tailings and prime starch. Exploratory fractionations with dilute formic, lactic, oxalic, and citric acids showed that these acids were about as effective as acetic. The cake flours yielded small amounts of tailings and gluten and a large amount of prime starch, compared with straight-grade flours of soft wheats. Glutens had 65 to 67% protein and 12% lipids, while prime starch fractions had under 0.2% protein and under 0.15% lipids. Water-solubles had from 13 to 16% protein, 0.4% lipids, and 4 to 5% ash.

Cake flour fractions could be successfully reconstituted by a method previously developed for cookie flours involving formation of a dough from the fractions, drying of the dough, grinding, and rehydration. In baking layer cakes it was possible to introduce the reconstituted dough directly into the baking test, thus eliminating drying of the dough, grinding, and rehydrating. This simplified procedure resulted in layer cakes that approached closely in scores and volumes the cakes baked from the corresponding original flours. Angel-food cake baking required the full reconstitution to a flour by drying the dough formed, grinding, and rehydrating. The reconstituted flours gave angel-food cakes matching closely the volumes of the cakes from the original flours. Good white layer cakes were obtained from all fractionations with other acids than acetic, but only citric acid fractionation resulted in yellow layer cakes comparable with those from acetic acid fractionations.

This paper deals with the development of fractionation and reconstitution techniques for cake flours to determine which flour fractions are affected by chlorine bleaching. It is generally assumed that chlorine bleaching destroys normal gluten characteristics and reduces pH (6). However, Alexander (1) pointed out that increased hydration capacity of chlorinated starches may also be an important factor. Most reports describe changes that occurred, or merely speculate on the con-

[1] Manuscript received March 25, 1957. Contribution from the Western Wheat Quality Laboratory, Department of Agricultural Chemistry, Washington Agricultural Experiment Stations, State College of Washington; and the Field Crops Research Branch, Agricultural Research Service, U.S. Department of Agriculture. Scientific Paper 1577, Washington Agricultural Experiment Stations, Pullman.

[2] Chemist, Western Wheat Quality Laboratory, Field Crops Research Branch, Agricultural Research Service, U.S. Department of Agriculture, Pullman, Wash.

stituents affected. Reliable quantitative evidence is lacking. Fractionation and reconstitution techniques could be used to determine the affected fractions.

Only a few reports concerning the application of these techniques to soft wheat flours have appeared. Yamazaki (13, 14) was able to fractionate soft wheat flours by modifying conventional dough-kneading procedures. He reconstituted the fractions for cookie baking by forming a dough from the fractions, then obtaining a flour from the dough by lyophilizing, grinding, and rehydrating. Sollars (10, 11) developed a fractionation procedure for low-protein flours that depended on acetic acid extraction of gluten rather than kneading. He applied this procedure to the study of cookie quality, first confirming Yamazaki's findings (13) that a simple blend of dry fractions yielded poor cookies, and then using a reconstitution method slightly modified from that of Yamazaki's to evaluate flour fractions for cookie quality. Zaehringer, Briant, and Personius (15) used dough-kneading methods to separate both hard and soft wheat flours in their study of biscuit quality. They were able to bake biscuits directly from a simple blend of dry fractions. Cake flour has been successfully fractionated and reconstituted, although no published reports exist of this work.[3]

The procedures previously developed for cookie flours appear to be well adapted to cake flours. Bleached cake flours represent perhaps the extreme limits of "softness" and "weakness" in flours, but acetic acid fractionation could be used to separate these flours since this process is independent of the amount or condition of gluten. The reconstitution procedure that yielded a flour suitable for cookie baking was modified for use in cake-baking studies. This paper reports the necessary modifications and some of the results.

Materials and Methods

A commercial cake flour milled from western soft wheats was used. One lot was bleached to an initial pH of 4.9 by a commercial bleaching agent consisting of chlorine with a small percentage of nitrosyl chloride, and the other lot was unbleached.

Analytical Methods. Moisture, ash, and protein analyses and pH determinations were made by conventional methods (2, 7). Lipid content was determined by the method of Mecham and Mohammad (8). Particle-size measurements were made by the sedimentation method of Hamelynck (5). In this method flour was added to a petroleum ether-acetone mixture in a glass tube, shaken for 1 minute, and allowed

[3] Waitman, R. H. (Central Laboratories, General Foods Corporation, 11th and Hudson Streets, Hoboken, N. J.); private communication.

to stand. The height of sediment was measured every minute for 10 minutes.

Flour Fractionation. The unbleached flour was separated into several fractions by a modification of the acetic acid fractionation (10).

To obtain the water-solubles, 600 g. flour were added to 1800 ml. distilled water, and the suspension was stirred for 10 minutes and then centrifuged for 10 minutes. The supernatant was decanted, filtered through glass wool, and concentrated under partial vacuum below 30°C. to a volume of 300 ml. The concentrate was removed, apparatus rinsed, and washings added to the concentrate until a volume of 390 ml. (65 ml. for each 100 g. flour) was reached. The liquid extract was then stored at 3°C. until needed.

Gluten was obtained by transferring the dough, one-third at a time, from the centrifuge bottles to a Waring Blendor cup and adding 500 ml. acetic acid solution of sufficient strength (usually 4 ml. glacial acetic acid and 496 ml. distilled water) to produce a pH of 3.5. The suspension was agitated for 1 minute and centrifuged for 10 minutes. The acid extract was decanted into a beaker. The residue was again extracted with acetic acid solution of sufficient strength (usually 0.5 ml. glacial acetic acid and 300 ml. distilled water) to maintain a pH of 3.5. After another centrifuging and decanting, the combined acid extracts were neutralized with 5*N* sodium hydroxide to pH 6.4.

Acid extracts of unbleached flour made at pH 3.5, when neutralized to pH 6.4, gave gluten precipitates that settled rapidly and could easily be recovered by decanting the supernatant. This gluten could be handled readily and cut up with scissors. Centrifugation of the supernatants yielded additional small amounts of gluten. All these glutens were cut into thin strips and air-dried.

The crude starch residue remaining after two acid extractions was resuspended in water. This was the third resuspension after the initial flour-water suspension. The pH of this third resuspension was adjusted from about 3.6 to 5.8 by 0.5*N* sodium hydroxide, and the suspension centrifuged for 10 minutes. The supernatant was discarded, and the tailings or upper layer of the residue was removed with a spatula, divided into thin strips, and air-dried.

The prime starch (lower layer) was resuspended in water, and the suspension poured into a Büchner funnel. The water was removed by suction, and the moist cake was cut into thin strips and air-dried.

Bleached cake flour required several further modifications of the acetic acid extraction process to effect successful fractionations and reconstitutions. The initial suspension of water and bleached flour was adjusted from pH 4.8 to pH 6.0 by 0.5*N* sodium hydroxide. The

suspension was then treated as previously described to obtain the water-solubles as a liquid concentrate of 65 ml. volume for each 100 g. flour.

Acid extracts of bleached flour were first neutralized to pH 5.0 (so that the dried gluten would have about this pH) and centrifuged to recover most of the gluten. Further neutralization of the supernatants to pH 6.4 and centrifugation yielded small amounts of gluten. All glutens failed to settle to a compact layer, were sticky, and could not be cut up with scissors. They were divided into thin strips and air-dried.

The crude starch residue remaining after two acid extractions of bleached flour was resuspended in water and centrifuged. (No pH adjustment was made for this third resuspension so that the pH of these fractions would remain at 5.0 or below.) Tailings and prime starch were recovered as previously described.

All dry fractions were ground on a Hobart coffee grinder, Model No. 2044, at the closest possible setting, and passed through a 35-mesh screen.

Reconstitution. For layer cake baking, a simplification of the reconstitution method developed by Yamazaki (13) and further modified by the author (11) was found to be feasible. The fractions were blended in the same proportions found in the original flour. Each sample contained the equivalent of 70 g. of fractions at 14% moisture. Sufficient water was added to the sample to bring the fractions to 14% moisture and furnish an additional 45.5 ml. (65% absorption). Nearly all of this water was furnished by the liquid concentrate. The blend and water were mixed with a National Manufacturing Co. 100-g. loaf mixer until a smooth, well-developed dough was formed. This dough was then used in the baking test without drying.

Full reconstitution to a flour was necessary for angel-food cakes, since methods of baking these cakes would not tolerate introduction of a dough. The equivalent of 55 g. of fractions was mixed as before to obtain a smooth dough which was then lyophilized. The dry material was ground in the Hobart coffee grinder at the closest possible setting, passed through a 35-mesh screen, and rehydrated to 14% moisture.

Cake-Baking Methods. The method employed in testing for layer cake baking quality was to add the flour or dough to a commercial cake mix minus flour and then bake cakes from this blend. The composition of the commercial cake mixes is given in Table I.

For baking white layer cakes from normal flours, samples were prepared by blending 70 g. flour with 117 g. cake mix, minus flour, for 5 minutes at low speed on a Hobart mixer, Model N-50. The

TABLE I
FORMULAS OF LAYER CAKE MIXES

Ingredients	White Cake Mix		Yellow Cake Mix	
	Percent	Percentage Based on 100% Flour	Percent	Percentage Based on 100% Flour
Sugar	42.93	115.0	43.73	120.0
Flour (cake grade)	37.34[a]	100.0	36.45[a]	100.0
Shortening (emulsified)	13.00	34.8	13.00	35.7
Milk (powdered)	4.75	12.7	4.75	13.0
Salt	0.70	1.9	0.70	1.9
Baking soda	0.54	1.4	0.58	1.6
Monocalcium phosphate	0.54	1.4	0.63	1.7
Sodium acid pyrophosphate	0.20	0.5	0.16	0.4
Total	100.00		100.00	
Flavoring	75 g. per 20 lb. mix		50 g. per 20 lb. mix	
Yellow dye			15 g. per 200 lb. mix	

[a] Flour was omitted from the mixes furnished.

mixture was stored in cans until needed. Just before baking, a supply of egg whites was prepared by mixing one part of dried egg whites with four parts of water. Water (35 ml.) was added over a 1-minute period at low speed to the flour-cake mix sample in the Hobart bowl, and the batter was mixed for 2 minutes at medium speed. Egg white preparation (21 g.) was blended in over a 1-minute period at low speed, and the batter mixed 1 minute at medium speed. Then 35 ml. of water were added over a 1-minute period at low speed, and the batter mixed 1 minute at medium speed. Batches of 230 g. of batter were weighed into a 6-in. layer pan and baked for 23 minutes at 365°F. Cakes were removed from pans while slightly warm, allowed to cool for 1 to 2 hours on an open bench, stored overnight in a closed cabinet, and were measured and scored the next day.

The procedure for reconstituted doughs was almost identical. The dough, containing 45.5 ml. water, was added to the cake mix in the bowl. The two materials were mixed for 1 minute at low speed and 2 minutes at medium speed. Egg-white preparation (21 g.) was blended in as before. Water (25 ml.) was added over a 1-minute period at low speed, and the batter mixed for 1 minute at medium speed. Cakes were then baked as before.

Baking of yellow layer cakes required only minor modifications of this basic procedure. For flours, 70 g. were mixed with 117 g. of yellow cake mix, minus flour. A supply of whole eggs was prepared just before baking by mixing one part of dried whole eggs with three parts of water. The sample was then mixed and baked according to the basic procedure, except that 33 g. of whole-egg preparation were

substituted for egg whites. Yellow cakes from reconstituted doughs were obtained by adding the dough to yellow cake mix, minus flour, and blending for 1 minute at low speed and 2 minutes at medium speed. Whole-egg preparation (33 g.) was blended in over a 1-minute period at low speed, and the batter mixed for 2 minutes at medium speed. Water (25 ml.) was added over a 1-minute period at low speed, and the batter mixed for 1 minute at medium speed. Cakes were baked from the batter by the basic procedure.

Angel-food cakes were baked according to procedures developed by Barmore (3). Sufficient egg whites for one day's bake were allowed to thaw at room temperature. Just before baking, the temperature of the egg whites was adjusted to 25°C. by immersing the beaker in a warm water bath and constantly stirring the egg whites. For each sample, 45 g. of the flour under test, 139 g. sugar, 4 g. potassium bitartrate, and 1 g. sodium chloride were blended for 5 minutes at low speed on the Hobart mixer, and the mixture was stored in a can until needed.

Egg whites (210 g.) were beaten for 2 minutes at medium speed and 1 minute at high speed on the Hobart mixer. The dry mixture was divided into four equal parts, and one part at a time was sifted over the beaten egg whites and blended for 10 seconds at low speed. After each blending, the bowl was scraped down. An additional 10 seconds' blending (20 seconds' total) was made after addition of the fourth portion. The batter was divided and 175 g. were placed in each of two tubeless pans 6 in. in diameter by 2½ in. deep. Cakes were baked for 30 minutes at 375°F., cooled 1 hour in an inverted position, removed from pans, and stored overnight in a closed cabinet.

Cake Measuring and Scoring Methods. After overnight storage, volumes of layer and angel-food cakes were measured by seed displacement. Layer cakes were scored by a method developed especially to rate the effect of chlorine bleaching on cake flours. The chart used in this method is presented in Table II along with typical scores for cakes from normal flours and reconstituted doughs.

Results and Discussion

Table III lists the yields and analytical data for the fractions. Portions of the water-soluble fractions were lyophilized to obtain these data.

Water Extraction of Bleached Flour. If a simple water extraction of bleached flour was made, as much as 40% of the total flour protein appeared in the water-solubles with a corresponding reduction in yield of gluten. When pH of the flour-water suspension was adjusted to 6.0

TABLE II

CAKE SCORING CHART AND TYPICAL RESULTS

FLOURS	IDEAL SCORE						TOTAL SCORE
	Crust	Symmetry	Silkiness	Tenderness	Grain	Color	
	15	5	5	10	35	30	100
	White layer cakes						
Unbleached							
Normal flours	8	3½	2½	6	18	20	58
Reconstituted	6	3	1	4	12	18	44
Bleached							
Normal flours	11	4	4	9	28	28	84
Reconstituted	10	4	4	9	28	27	82
	Yellow layer cakes						
Unbleached							
Normal flours	½	½	½	½	2	18	22
Reconstituted	1	1	½	½	2	15	20
Bleached							
Normal flours	11	4	4½	9	29	28	85½
Reconstituted	9	4	4	8½	27	27	79½

for this extraction, a normal water-soluble fraction was obtained as shown in Table III. Provided this pH adjustment was made for bleached flours, no particular difficulties were encountered in fractionation of cake flours. All other modifications of the process were made to improve the quality of the reconstituted products.

TABLE III

YIELDS, ANALYSES, AND PHS OF ORIGINAL FLOURS, FRACTIONS, AND RECONSTITUTED FLOURS

MATERIAL	YIELD[a]	PROTEIN[b]	LIPID[b]	ASH[b]	pH
	g.	%	%	%	
Unbleached flour					
Original flour	100.0	6.04	1.20	0.34	5.82
Fractions					
Water-solubles	3.9	16.1	0.36	4.06	...
Gluten	5.7	67.0	12.1	0.71	6.17
Tailings	8.3	5.51	1.65	0.32	5.88
Prime starch	78.7	0.13	0.10	0.28	5.92
Recovery	96.6	83.3	76.8	...	...
Reconstituted flour	...	6.39	1.40	0.31	5.96
Bleached flour					
Original flour	100.0	6.21	1.25	0.34	4.82
Fractions					
Water-solubles	3.4	13.4	0.34	5.12	...
Gluten	5.9	67.3	11.5	0.58	5.10
Tailings	8.7	4.00	1.30	0.38	4.12
Prime starch	78.6	0.18	0.12	0.24	5.01
Recovery	96.6	78.7	71.6	...	...
Reconstituted flour	...	6.09	1.28	0.30	5.21

[a] Grams at 14% moisture from 100 g. flour at 14% moisture.
[b] At 14% moisture.

Effect of Lyophilizing Water-Solubles. Lyophilization of the water-soluble fraction to a dry material was discontinued following reports by Shogren and Finney[4] that this treatment caused some damage to loaf volume in bread-baking studies. Only when they returned to the use of water-solubles in the original liquid state were they able to match the loaf volume of their original flour.

In a brief investigation of the above reports, cookie flour was fractionated, and the water extract was thoroughly mixed. One part of this extract was lyophilized, and the other part was stored as a liquid concentrate. Cookies baked from flour reconstituted with liquid concentrate proved to be slightly better than those baked from flour reconstituted with dried water-solubles. An additional advantage of the liquid concentrate was the time saved by elimination of lyophilization. Storage and use of the water extract as a liquid concentrate was then adopted for the cake-baking study. Usual storage did not exceed 3 days from the time of separation until incorporation into a dough.

Effect of pH Level of Individual Fractions. In the original acid extraction process (10), the acid extract was neutralized to pH 6.4, and the starch residue remained at a pH of about 3.6 when wet. Fractions thus obtained had the following pH values whether from bleached or unbleached flour: gluten, 6.2 to 6.3; tailings, 4.0 to 4.1; and prime starch, 4.6 to 5.0. Doughs were reconstituted from liquid water-solubles and these fractions in proportions of the original flours; portions of these doughs when lyophilized and ground to a flour had pH values of 5.4 to 5.6 whether the fractions were from bleached or unbleached flour.

Doughs reconstituted entirely from fractions obtained from bleached flour under these conditions yielded cakes that were close to the cakes from normal bleached flour. Typical results were:

	White Layer Cakes		*Yellow Layer Cakes*	
	Scores	*Volumes*	*Scores*	*Volumes*
Normal bleached	86	580	86	620
Reconstituted bleached	80	580	80	640

Doughs reconstituted entirely from fractions obtained from unbleached flour under these conditions yielded cakes with the following typical results:

	White Layer Cakes		*Yellow Layer Cakes*	
	Scores	*Volumes*	*Scores*	*Volumes*
Normal unbleached	53	530	22	340
Reconstituted unbleached	54	590	50–70	610

The white layer cakes from reconstituted unbleached doughs were

[4] Shogren, M. D., and Finney, K. F. (Hard Winter Wheat Quality Laboratory, Manhattan, Kansas). Unpublished results.

often slightly better than those from normal unbleached flours. The yellow layer cakes from reconstituted unbleached doughs were much better than those from normal unbleached flour and frequently approached such cakes from reconstituted bleached doughs. The reconstituted unbleached doughs thus gave better cakes than those obtained from normal unbleached flours, and this was caused primarily by the low pH (4.0 to 5.0) of the tailings and prime starch.

Adjustment of Fraction pH. The fractionation procedure was then modified to yield fractions of about pH 6.0 from unbleached flour and about pH 5.0 from bleached flour. This was accomplished for unbleached flour by resuspending the crude starch residue (the third resuspension) and adjusting the pH to 5.8. For bleached flour, the acid extract was first neutralized to pH 5.0, and most of the gluten was recovered at this pH. Other fractions were left at pH levels obtained in the original process. Water-solubles from bleached flour had been adjusted to pH 6.0 at commencement of fractionation. The pH of this fraction was readjusted to 5.0 at time of reconstitution. Thus the following pH values were obtained:

	Bleached Flour	*Unbleached Flour*
Gluten	5.1	6.2
Tailings	4.1	5.9
Prime starch	5.0	5.9
Flour reconstituted from above fractions and liquid solubles	5.2	6.0

Doughs reconstituted entirely from fractions of unbleached flour and with the pH so adjusted gave yellow layer cakes with scores of about 20 and volumes of about 360 ml. and white layer cakes with scores of about 44 and volumes of about 510 ml. Doughs reconstituted entirely from fractions of bleached flour and with the pH so adjusted gave cakes with very little change in score and volumes from results previously obtained. The reconstituted products were considered satisfactory for fraction interchanges.

For fractions from unbleached flour, the only changes in pH were for tailings and prime starch; for fractions of bleached flour, the only change in pH was for gluten. Since little change occurred in quality of cakes from reconstituted bleached doughs, but a large change occurred in quality of cakes from reconstituted unbleached doughs, it appears that pH level is much more critical for bleached starch than for bleached gluten.

Third Resuspension for Starch Residues from Bleached Flour. If tailings and prime starch were obtained from the residue after two acid extractions of bleached flour, the pH of these fractions was satis-

factory. Yields were: tailings, 14%; and prime starch, 73%. To obtain the corresponding fractions with a pH of 6.0 from unbleached flour, however, the residue after two acid extractions had to be resuspended (the third resuspension), pH adjustment made, and the suspension centrifuged. This third resuspension and centrifugation of the residue from unbleached flour effected a further concentration of tailings and resulted in yields of 8% tailings and 78% prime starch. To facilitate interchange of fractions, the residue from two acid extractions of bleached flour was resuspended a third time without any pH adjustment and centrifuged to concentrate the tailings; yields were 9% tailings and 78% prime starch.

Relative Yields of Fractions from Cake Flour. The cake flour studied had a higher yield of prime starch and lower yields of tailings and gluten as compared with straight-grade flours from low-protein wheats. Prime starch yields of 62 to 65% were obtained by acid fractionation of straight-grade flours of soft wheats (10); some flours from club wheats gave as high as 69% prime starch. In the present study two resuspensions of cake flours gave yields of 73% prime starch. Current practice is to select mill streams with low ash content for cake flours. These experiments indicate that the content of prime starch might be a valuable test for a cake flour or the suitability of a mill stream for cake flour.

Baking Properties of Simple Blends of Dry Fractions. Attempts to use a reconstituted "flour" made by assembling a simple blend of dry fractions in correct proportions invariably resulted in extremely poor cakes. Even long mixing during the baking test, of the stiff batter formed after the first addition of water, failed to achieve satisfactory dough development. Similar attempts in cookie flour reconstitutions were also unsuccessful (13). Formation of a dough from the blended fractions and water before addition of other ingredients appears to be essential. This is a distinctly different requirement from that for flour reconstitutions for baking yeast-leavened products in which a simple blend of dry fractions suffices. Yet Zaehringer, Briant, and Personius (15) apparently used a simple blend of dry fractions for biscuit baking, which depends on chemical leavening agents. Although they did not develop their blends of fractions into doughs by mechanical mixing before adding the final ingredients, they did form a dough by mixing with 40 strokes by hand using a limited amount of water (46% for soft wheat reconstituted flours). This partial mixing at a low water level may have sufficed to develop the gluten.

Particle-Size Distribution of Reconstituted Cake Flours. The importance of particle size to cake quality frequently has been demon-

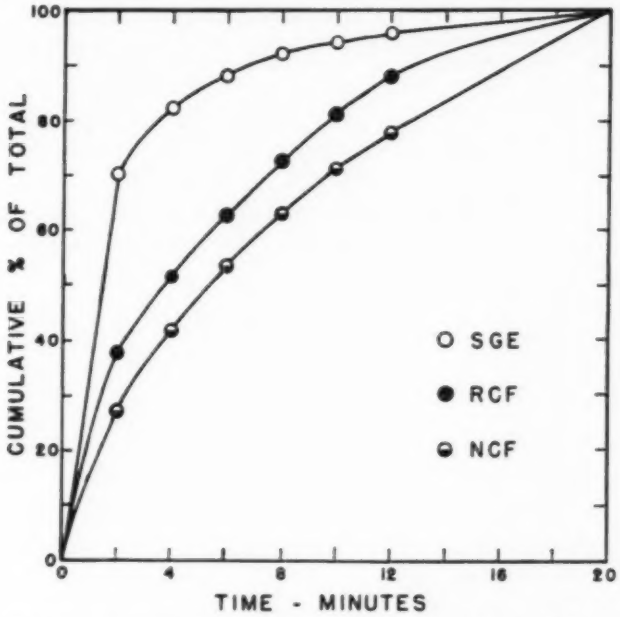

Fig. 1. Particle size determinations: NCF, normal cake flour; RCF, reconstituted cake flour; SGE, straight-grade Elgin flour included for comparison.

strated (9). A brief investigation of particle-size distribution of reconstituted cake flours was undertaken in this study for comparison with that of normal cake flours. Figure 1 shows the results. The particle-size distribution of reconstituted flour was very close to that of the original cake flour and was much finer than that of experimentally milled, straight-grade flours. No significant difference in distribution patterns was found between unbleached and bleached flours.

Use of Other Acids Than Acetic for Gluten Extraction. Cunningham, Geddes, and Anderson (4) have prepared cohesive proteins by extracting wheat, barley, and rye flours with dilute formic, oxalic, and citric acids. Dilute lactic acid has been employed to disperse wheat gluten, and the recovered gluten suffered very little damage, as shown by bread-baking tests.[5] Routine fractionations of bleached cake flour were made with each of the above acids substituted for acetic acid. A pH of 3.5 was obtained in all acid extractions by adding varying amounts of acid. Enough water was added to maintain a ratio of 500 ml. solvent to 200 g. (starting weight) of flour. Only two resuspensions of the residue were made. Table IV gives results of the fractionations. The other acids appeared to be about as effective as acetic in

[5] Finney, K. F. (Hard Winter Wheat Quality Lab., Manhattan, Kansas). Unpublished results.

TABLE IV

MATERIALS BALANCES FOR TYPICAL ACID FRACTIONATIONS OF A BLEACHED CAKE FLOUR

ACID (DILUTE) USED TO EXTRACT GLUTEN	FRACTION	YIELD[a]	PROTEIN[b] CONTENT	DRY MATTER RECOVERY	TOTAL FLOUR PROTEIN RECOVERED IN FOUR FRACTIONS	TOTAL FLOUR PROTEIN NOT PRECIPITATED FROM NEUTRALIZED EXTRACTS
		g.	%	%	%	%
Formic	Water-solubles	3.4	[c]	96.1	82.9	11.2
	Gluten	5.4	63.6			
	Tailings	12.8	2.72			
	Prime starch	74.5	0.23			
Lactic	Water-solubles	3.5	[c]	95.7	84.0	12.0
	Gluten	5.1	62.9			
	Tailings	13.1	2.39			
	Prime starch	74.0	0.20			
Oxalic	Water-solubles	3.4	[c]	95.8	78.6	17.4
	Gluten	5.0	66.3			
	Tailings	13.4	3.26			
	Prime starch	74.0	0.22			
Citric	Water-solubles	3.6	[c]	95.6	78.8	17.9
	Gluten	4.8	64.1			
	Tailings	14.7	2.61			
	Prime starch	72.5	0.19			
Acetic	Water-solubles	3.4	[c]	96.2	83.2	
	Gluten	5.5	66.8			
	Tailings	13.9	3.03			
	Prime starch	73.4	0.20			

[a] Grams at 14% moisture from 100 g. flour at 14% moisture.
[b] At 14% moisture.
[c] Protein content of liquid extracts determined.

extracting gluten, but somewhat more protein remained soluble in neutralized extracts from oxalic and citric extractions, with a corresponding reduction in amount of total flour protein recovered in the fractions.

Layer cakes were baked from these fractions. Table V lists the

TABLE V

CAKE SCORES AND VOLUMES OBTAINED WHEN DIFFERENT ACIDS WERE USED TO EXTRACT GLUTEN

	NORMAL BLEACHED FLOUR	ACID USED: RECONSTITUTED FLOUR				
		Dilute Acetic	Dilute Formic	Dilute Lactic	Dilute Oxalic	Dilute Citric
White layer cakes						
Scores	86	81	81	83	82	81
Volumes (ml.)	580	580	580	560	590	590
Yellow layer cakes						
Scores	85	80	69	67	68	79
Volumes (ml.)	695	670	675	600	610	675

scores and volumes of these cakes. All dilute acids gave good white layer cakes, but only citric acid gave yellow layer cakes comparable with those from acetic acid.

Cake-Baking Results. Table VI gives results of baking layer cakes from doughs reconstituted from fractions of the acetic acid process

TABLE VI

AVERAGE SCORES AND VOLUMES FOR CAKES FROM NORMAL FLOURS AND RECONSTITUTED DOUGHS

	SCORES	STD. ERROR OF THE MEAN	VOLUMES	STD. ERROR OF THE MEAN
			ml.	
	White layer cakes[a]			
Unbleached				
Normal flours	56	1.7	583	8.8
Reconstituted doughs	44	1.4	512	7.3
Bleached				
Normal flours	84	0.4	580	2.9
Reconstituted doughs	80	1.0	577	6.7
	Yellow layer cakes[a]			
Unbleached				
Normal flours	22	0.4	340	7.6
Reconstituted doughs	20	2.9	368	6.1
Bleached				
Normal flours	86	0.0	620	0.0
Reconstituted doughs	78	1.0	605	5.0
	Angel-food cakes[a]			
Unbleached				
Normal flours			606	9.5
Reconstituted flours[b]			567	3.4
Bleached				
Normal flours			699	21.3
Reconstituted flours[b]			713	18.8

[a] Average results for three series of reconstitutions with the type of cake described.
[b] For angel-food cake baking, reconstitution to a flour was used.

and angel-food cakes from flours reconstituted from fractions of this process. Most of the reconstituted cakes were close enough to the normal cakes to be considered satisfactory. One exception was the reconstituted unbleached white layer cakes which had a score of only 44 compared with a score of 56 for the normal unbleached white layer cake.

One surprising result was that the volume of the white layer cakes from unbleached flour was either almost as great as, or was fully equal to, the volume of the cake from bleached flour. For the three series of bakes given in Table VI, the cake from normal unbleached flour had 100.5% of the volume of the cake from normal bleached flour. Previously, for six different bakes over a period of 4 months,

the normal unbleached cake averaged 93% of the volume of the normal bleached cake; standard errors of a single determination were 12 ml. and 8 ml. respectively.

Most workers have reported greater differences in volume for this type of cake from unbleached and bleached flours. In a brief study of this anomaly, different bakers were used, another pair of commercial cake flours and three pairs of single-variety cake flours milled and bleached in this laboratory were baked into cakes, and 8-in. pans plus larger size batters were used. Only the use of 8-in. pans caused any lowering of volume; with these pans and commercial flours the unbleached cakes had from 87 to 91% of the volume of bleached cakes. However, the unbleached portions of the single-variety cake flours prepared here, when baked in 8-in. pans, gave cake volumes from 95% to 101% of the corresponding bleached flours.

As with cookie flour fractionations and reconstitutions, the reconstituted product usually did not quite match the quality of the product from normal flour. Slight or obvious differences were usually evident. Walden and McConnell (12) have pointed out that doughing of flour damaged the baking quality of bread, but damage to baking quality was negligible when they used 1.75% sodium chloride in the water.

However, the white layer cake reconstituted from all bleached fractions had an average score of 80 compared with an average score of 84 for the cake from normal bleached flour, and it frequently was very difficult to distinguish between the two. Table II gives an example of an individual reconstituted bleached white layer cake that was very close to the cake from normal bleached flour – scores of 82 against 84. The high quality of white and yellow layer cakes reconstituted from bleached fractions is fortunate, since it is anticipated that most studies of cake quality will deal with bleached flours.

The procedures reported in this paper were used in preliminary studies of cake flour bleaching and will be used for other investigations of cake flour quality. The substitution of reconstituted doughs for reconstituted flours in layer cake baking has been found to be especially helpful, since this substitution eliminated lyophilization, grinding of doughs, rehydration, and redetermination of moisture content. Thus, a shorter, more simple reconstitution procedure with fewer variables was made possible. It is hoped that further improvements can be made so as to match the cakes from normal flours more closely.

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CAKE AND COOKIE FLOUR FRACTIONS AFFECTED BY CHLORINE BLEACHING[1]

WILLIAM F. SOLLARS[2]

ABSTRACT

Unbleached and bleached cake and cookie flours were separated into water-solubles, gluten, tailings, and prime starch by a modified acetic acid fractionation procedure. The flours were bleached with a commercial bleaching agent consisting largely of chlorine with a small percentage of nitrosyl chloride. Reconstitutions were made in which one fraction at a time was interchanged between blends of unbleached fractions and blends of bleached fractions.

In mixing to a dough during reconstitution, blends of all unbleached fractions required a much longer blending time than did blends of all bleached fractions. The tests of interchanged fractions showed that this difference in blending time was caused by differences in the gluten and prime starch.

For white layer cakes, all of the improvement effected by bleaching occurred in the gluten and prime starch in about equal proportions. The large response to chlorine bleaching shown by yellow layer cakes appeared to occur almost entirely in the prime starch. However, there was a small response from gluten interchanges. Increased volumes of angel-food cakes from bleached flours were effected about equally by gluten and prime starch, with a small improvement caused by water-solubles. For chlorine-bleached cookie flours, major responses in cookie diameter were consistently due to gluten and prime starch; other fractions gave only occasional, small responses.

All fractions contributed to the drop in pH, but gluten caused 50% of the drop while prime starch caused only 10%. The effect on pH was roughly proportional to the logarithm of the amount of protein in the interchanged fraction.

A rapid, simple cookie-baking test (4) has been of great value in rating varieties of soft wheat for baking quality. A similar test for evaluating cake-baking quality would be equally valuable, and several attempts have been made to devise one. However, the development of such a test has been hampered by lack of fundamental knowledge of cake flours. One problem encountered is determination of the optimum bleach level.

Practically all cake flour today is bleached with chlorine or with Beta Chlora in which chlorine is the principal ingredient.[3] This agent

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[3] U.S. Patent 1,096,480, granted to J. A. Wesener, specified a mixture of chlorine and nitrosyl chloride with not more than 5% of the latter (Official Gazette of the U.S. Patent Office, Vol. 202, p. 481; May 12, 1914). Usually only 0.5 to 1.0% nitrosyl chloride is present. Bailey in 1925 gave the composition as 0.5% nitrosyl chloride and 99.5% chlorine (Bailey, C. H. The chemistry of wheat flour, p. 217. The Chemical Catalog Co., Inc.: New York [1925]). Geddes and Dunlop in 1949 also gave the composition as 0.5% nitrosyl chloride and 99.5% chlorine (Kirk, R. E., and Othmer, D. F. Encyclopedia of chemical technology, Vol. 3, p. 622. Interscience: New York, 1949).

has a unique effect. There is a sharp drop in flour pH, and cakes with a higher ratio of sugar and shortening to flour can be produced. Cakes from bleached flour are more tender and are less likely to fall. They have finer grain, improved color, and often increased volume (3, 5, 7). Yet very little is known about the fundamental chemistry of chlorine bleaching. Most references describe the changes or merely speculate upon the causes.

The reduction in pH is well known, and it is generally assumed that normal gluten characteristics are destroyed (6). Bohn (3) stated that gluten was made more elastic and therefore capable of forming thinner films. Harrel (5) distinguished between the true bleaching and maturing actions of chlorine on cake flours and considered the maturing action to have an important effect. Harrel believed that the greater number of gas cells in cake from bleached flours improved the texture and grain. Alexander (1) appears to be the only one who considered the action of chlorine on starch. He pointed out that chlorination causes both increased dispersion of gluten and increased hydration capacity of starch and gluten. Quantitative evidence, however, appears to be lacking.

At present, the level to which a flour is bleached[4] depends either on trial-and-error methods or on previous experience with flours of similar grade and ash and protein content. For bleaching commercial flours, the amount of chlorine recommended is proportional to flour ash and protein.[5] A common practice in both mill and research laboratories is to bleach a flour to a number of pH levels and bake cakes from each level to determine which pH gives the optimum results. There does not appear to be any rapid, fundamental method for determining the optimum bleach level. The difficulties of developing a cake-baking test under such procedures are apparent.

In a study of the chlorine bleaching process, the first phase, reported here, was to use fractionating and reconstituting techniques to locate the fractions affected. Since there is a decided difference between cakes from bleached flour and those from unbleached flour, reconstitutions made with one fraction interchanged at a time, according to the techniques employed by many investigators, should show which fractions were affected. Chlorine bleaching also affects cookie-baking quality by lowering cookie diameter and changing top grain. The same techniques were used to determine affected fractions in cookie flours for comparison with affected fractions in cake flours.

[4] It is recognized in agreement with Harrel (5) and others that the primary improvement caused in cake flours by chlorine is not an improvement in color (bleaching) but rather is the improvement in cake texture, grain, and volume (maturing action). In keeping with common usage, however, this maturing action will be called "bleaching" in this paper.

[5] Harris, M. C. (National Biscuit Co., 428 W. 16th St., New York 11, N. Y.); private communication.

Materials and Methods

The bleached and unbleached cake flours have been described (9). The cookie flours were straight-grade, single-variety flours milled on the Buhler mill, and contained about 8% protein. A portion of each flour was treated with Beta Chlora in a laboratory-size apparatus until a pH of 5.0 to 5.2 was reached.

Another paper reports fractionation of cake flours, analyses of flours and fractions, and methods of reconstitution, cake baking, and scoring (9). For layer cake baking, reconstitution to a dough was used. Baking of angel-food cakes required full reconstitution to a flour of about 14% moisture content.

Bleached and unbleached cookie flours were fractionated at pH 3.5 according to the procedures developed for cake flours (9). Full reconstitution to a flour of about 14% moisture was followed as previously reported (8). Cookies were baked by the micro baking procedure of Finney, Morris, and Yamazaki (4).

Results and Discussion

Effect of Bleaching on Reconstitution Blending Time. It has been shown that flour fractions for cookie and cake baking can be reconstituted successfully only by blending the fractions with water to form a dough of optimum development (9, 10, 11). The time required to develop this dough was termed reconstitution blending time to distinguish it from mixing time during the actual baking test. Blends of fractions, as well as the original flours, vary greatly in their water absorption, but they usually reflect that of the original flour. This complicates the interchange of fractions between two blends having widely different absorption levels. The procedure adopted in previous studies was to choose a uniform water absorption for all blends and allow blending time to vary so that optimum development occurred. In a study of cookie baking quality it was found that the fraction responsible for most of the quality difference also affected the blending time most (8).

At 65% absorption, blends made up entirely of fractions from bleached cake flour had a short blending time and formed firmer, stiffer doughs than did similar blends from unbleached cake flour. Table I shows the difference in blending time for these two blends and also lists the times for blends with one interchanged fraction. The reconstitution procedure for all types of cake baking was the same through the dough formation step, and the blending times were recorded for a large number of reconstitutions.

Bleached gluten and bleached prime starch shortened the blending time decidedly for the blends with one interchanged fraction in the

TABLE I

TIME REQUIRED TO MIX CAKE FLOUR FRACTIONS TO A DOUGH DURING RECONSTITUTION[a]

Unbleached Reconstituted Flours[b]			Bleached Reconstituted Flours[c]		
Flour No.	Time	Standard Error of the Mean	Flour No.	Time	Standard Error of the Mean
	Minutes			*Minutes*	
1. All unbleached fractions	11	0.6	6. All bleached fractions	4	0.3
2. Bleached water-solubles	11	0.3	7. Unbleached water-solubles	4	0.4
3. Bleached gluten	6	0.2	8. Unbleached gluten	8	1.1
4. Bleached tailings	10	0.0	9. Unbleached tailings	4	0.2
5. Bleached prime starch	5	0.5	10. Unbleached prime starch	9	0.9

[a] Average blending times for six independent reconstitution series, two each for white layer cakes, yellow layer cakes, and angel-food cakes.
[b] Flours 2 to 5 were similar to flour 1 except for the fraction mentioned.
[c] Flours 7 to 10 were similar to flour 6 except for the fraction mentioned.

cake flour reconstitutions and in Elgin and Brevor cookie-flour reconstitutions. Normal Rio straight-grade flour has a relatively high absorption, and at 65% absorption there was no difference in time required by blends of unbleached and bleached fractions for this variety. While the differences observed were recorded as differences in blending time, they probably were actually differences in water absorption and thus may lend support to Alexander's remarks about the increased hydration capacity of bleached starch (1).

White Layer Cake Baking. Table II presents scores and volumes for white layer cakes baked from unbleached and bleached normal cake flours, doughs reconstituted from blends in which all fractions were from one of these flours, and doughs reconstituted from blends in which one fraction was interchanged. Only gluten and prime starch caused the difference in quality (scores). When these two fractions were unbleached and were substituted in the blend of all bleached fractions, they were about equally effective in lowering the score. Bleached gluten, however, was significantly more effective in raising the score when substituted in the blend of all unbleached fractions than was bleached prime starch. There was no significant difference in volume between cakes from normal unbleached flour and normal bleached flour (583 and 580 ml.; standard errors of a single determination, 15.3 and 5.0 ml., respectively). The reconstituted unbleached cake had a volume of 512 ml. and a standard error of a single determination of 12.6 ml. compared with a volume of 577 ml. and a standard error of a single determination of 10.7 ml. for the reconstituted bleached cake; this was a

TABLE II

AVERAGE SCORES AND VOLUMES OF LAYER CAKES FROM NORMAL FLOURS AND RECONSTITUTED DOUGHS

	SCORES[a]		VOLUMES[a]	
	Unbleached	Bleached	Unbleached	Bleached
			ml.	*ml.*
	White layer cakes			
Original flours	56	84	583	580
Reconstituted flours				
All fractions from same treatment	44	80	512	577
Interchanged solubles	44	78	517	577
Interchanged gluten	66	66	515	587
Interchanged tailings	42	78	497	578
Interchanged prime starch	57	67	572	527
	Yellow layer cakes			
Original flours	22	86	340	620
Reconstituted flours				
All fractions from same treatment	20	78	368	605
Interchanged solubles	20	79	363	593
Interchanged gluten	20	72	360	603
Interchanged tailings	24	78	393	595
Interchanged prime starch	73	21	600	387

[a] Average scores and volumes for three independent series of reconstitutions for each type of cake listed. In each reconstitution series, one fraction at a time was interchanged between unbleached and bleached flours.

significant difference. All of this volume difference for reconstituted doughs was caused by the prime starch fraction and was true for both unbleached and bleached prime starches. For these cakes, however, most of the improvement from bleaching came in the better grain, color, tenderness, and crust.

The experiments with white layer cakes proved to be the most consistent of this study. Remarkable uniformity was achieved in scores and volumes for cakes with interchanged water-solubles and interchanged tailings as compared with corresponding cakes with all fractions from the same treatment. For this series of cakes the dough blending time could have been used to predict fractions responsible for quality differences. Reconstituted doughs from all unbleached fractions gave cakes lower in scores than desired. Those from all bleached fractions had quite satisfactory scores and volumes. Thus, the use of fractionating and reconstituting techniques to produce doughs for white layer cake baking was found to be relatively rapid, well adapted, and satisfactory.

Yellow Layer Cake Baking. The above series of normal cake flours and reconstituted doughs was subjected to the yellow layer cake test. The results (Table II) were clear; practically all improvement from

bleaching was located in the prime starch fraction. This fact was shown in two ways. When bleached starch was added to the blend with three other fractions unbleached, the score rose from 20 to 73, and the volume increased from 368 ml. to 600 ml. When unbleached starch was added to the blend with three other fractions bleached, the score dropped from 78 to 21 and the volume from 605 to 387 ml. The addition of unbleached gluten to the blend with three other fractions bleached caused a minor drop in score but not in volume. Addition of bleached gluten to the blend with three other fractions unbleached caused no change.

The unbleached flours gave yellow layer cakes of abnormally low volume, less than 400 ml. compared with 620 ml. for cakes from bleached flours. When first removed from the oven, cakes from unbleached flours were as large and had as good an appearance as those from bleached flours, but yellow cakes from unbleached flours fell drastically on cooling. The end result was a shallow layer, sunken in the middle, with soggy streaks and extremely poor grain and texture. This behavior was strikingly similar to that described by Bohn (3).

Bleached prime starch seemed to be essential for development of a normal structure in yellow layer cakes. If the normal structure were lacking, it is doubtful whether the addition of an agent with a potential for grain improvement would cause any appreciable improvement. This may explain failure of bleached gluten to show any improvement when it was substituted for unbleached gluten. Additional evidence that gluten had a small effect may be seen in the failure of bleached prime starch to raise the score by itself to the score of the reconstituted all-bleached cake. The substitution of bleached starch gave a score of 73 against 78 for the control. It was clear, however, that prime starch accounted for most of the improvement from bleaching for these cakes.

Baking tests with yellow layer cakes, coupled with fractionating and reconstituting techniques, may have as great a value in cake quality studies as have the cookie quality tests published in this issue (9). Both reproducibility of each of the twelve samples baked and uniformity of the entire series were very good over three series of independent reconstitutions. For the ten reconstituted samples for the three series, the coefficient of variation was 5.7% for scores and 2.6% for volumes.

Angel-Food Cake Baking. Table III lists volumes of angel-food cakes baked from normal flours and reconstituted flours. Most of the volume difference was caused by gluten and prime starch. The water-solubles appeared to contribute only a small effect. Substitution of bleached tailings for unbleached tailings caused an increase in volume

TABLE III

AVERAGE VOLUMES OF ANGEL-FOOD CAKES AND pH VALUES OF NORMAL AND RECONSTITUTED FLOURS

	VOLUME[a] OF ANGEL-FOOD CAKE		pH VALUE[a] OF FLOUR	
	Unbleached	Bleached	Unbleached	Bleached
	ml.	*ml.*		
Original flour	606	699	5.82	4.82
Reconstituted flours				
All fractions from same treatment	567	713	5.95	5.22
Interchanged solubles	585	706	5.71	5.32
Interchanged gluten	664	679	5.52	5.50
Interchanged tailings	604	729	5.79	5.36
Interchanged prime starch	665	687	5.88	5.31

[a] Average volumes and pH values for three independent series of reconstitutions in which one fraction at a time was interchanged between the two flours listed.

– 604 compared with 567 – but substitution of unbleached tailings for bleached also caused an increase in volume over the control, reconstituted, all-bleached cake – 729 compared with 713. This unexpected effect of unbleached tailings was observed in all three angel-food cake reconstitutions.

The angel-food cake baking test was the least satisfactory of the four baking tests employed in this study. The addition of unbleached and bleached flours to an angel-food cake mix minus the flour was attempted, but resulted in a standard error of a single determination of 58.4 ml. (average volume, 538 ml.) for five bakes with unbleached flour and a standard error of a single determination of 51.2 ml. (average volume, 634 ml.) for twelve bakes with bleached flour. Similar poor results were obtained with several other baking methods. All methods, including the one selected, gave occasional fallen cakes or cakes with widely differing volumes. For the method selected, the standard errors of a single determination were: normal unbleached cake, 16.5 ml.; reconstituted unbleached cake, 5.9 ml.; normal bleached cake, 36.9 ml.; and reconstituted bleached cake, 32.5 ml. For this method, for volumes of the ten reconstituted cakes for the three series, the coefficient of variation was 4.8% as compared with corresponding coefficients of 2.8 and 2.6% for white layer cakes and yellow layer cakes, respectively.

The average results from this test, however, indicated that gluten and prime starch again were mainly responsible for the improved quality from chlorine bleaching.

Baking data for all three types of reconstituted cakes were subjected to variance analysis, and the results are given in Table IV.

Cookie Baking. A distinct loss in cookie diameter and top grain

TABLE IV

ANALYSIS OF VARIANCE FOR BAKING DATA OF RECONSTITUTED CAKES FROM MEANS GIVEN IN TABLES II AND III

Source of Variance	Degrees of Freedom	Scores			Volumes		
		Sum of Squares	Mean Squares	Calculated F Values[a]	Sum of Squares	Mean Squares	Calculated F Values[a]
		White layer cakes					
Due to bleaching	1	4013	4013.7	389.7	16,334	16,333.7	69.5
Between fractions within bleaching	8	1786	223.3	21.7	16,803	2,100.4	8.9
Residual (error)	20	206	10.3	...	4,700	235.0	...
		Yellow layer cakes					
Due to bleaching	1	8501	8500.9	1104.0	146,301	146,300.9	897.6
Between fractions within bleaching	8	13,720	1715.0	222.8	236,323	29,540.4	181.2
Residual (error)	20	153	7.7	...	3,250	162.5	...
		Angel-food cakes					
Due to bleaching	1	(not scored)		...	55,212	55,212.0	55.8
Between fractions within bleaching	8	...	...	...	29,579	3,697.4	3.7
Residual (error)	20	...	...	...	19,796	989.8	...

[a] The F values for 1 and 20 degrees of freedom are: 5% point, 4.35, and 1% point, 8.10; and for 8 and 20 degrees of freedom: 5% point, 2.45, and 1% point, 3.56.

quality, caused by chlorine treatment of cookie flours, has been reported by others (1, 5). Since cookie-baking quality of a flour is measured directly by cookie diameter, chlorine bleaching does not improve cookie flours but is useful (5) in controlling cookie size to meet packaging specifications. The location of affected fractions of cookie flours was undertaken for comparison with results from cake flour experiments.

Three single-variety flours were selected, and a portion of each flour was bleached. Only a single series of reconstitutions was made with each pair of unbleached and bleached flours, as it was considered more desirable to have three varieties represented rather than make replications of a flour from one variety. Cookie diameters are given in Table V for the series with flours of Elgin, a club-wheat variety, and Brevor, a common soft-wheat variety. The major responses in both cases were from gluten and prime starch. Occasional, sometimes contradictory, small responses were shown by water-solubles and tailings. Top grain appeared to be affected mainly by gluten.

Table V also gives diameters obtained with bleached and unbleached flours of Rio, a common hard-wheat variety. Rio is considered as having poor soft-wheat quality because it gives cookies with small diameters and poor top grain. Nevertheless, the flour was affected by

TABLE V

DIAMETER OF COOKIES FROM UNBLEACHED AND BLEACHED FLOURS[a]

	UNBLEACHED ELGIN	BLEACHED ELGIN	UNBLEACHED BREVOR	BLEACHED BREVOR	UNBLEACHED RIO	BLEACHED RIO
	cm.	cm.	cm.	cm.	cm.	cm.
Original flours	9.29	8.57	9.83	8.62	7.99	7.71
Reconstituted flours						
All fractions from same treatment	9.00	8.31	9.60	8.69	7.95	7.66
Interchanged solubles	8.89	8.17	9.82	8.55	8.16	7.78
Interchanged gluten	8.59	8.45	9.19	9.10	7.68	7.88
Interchanged tailings	8.99	8.31	9.33	8.52	8.00	7.59
Interchanged prime starch	8.55	8.86	9.11	8.91	7.89	7.96

[a] Cookie diameters for a single reconstitution series with bleached and unbleached straight-grade flours of the varieties listed. One fraction at a time was interchanged between bleached and unbleached flours of the same variety.

chlorine. Cookie diameter, although small, showed a small drop after bleaching. Because Rio is a hard-wheat variety, a reconstitution series was made with this flour to determine if the same fractions were affect-

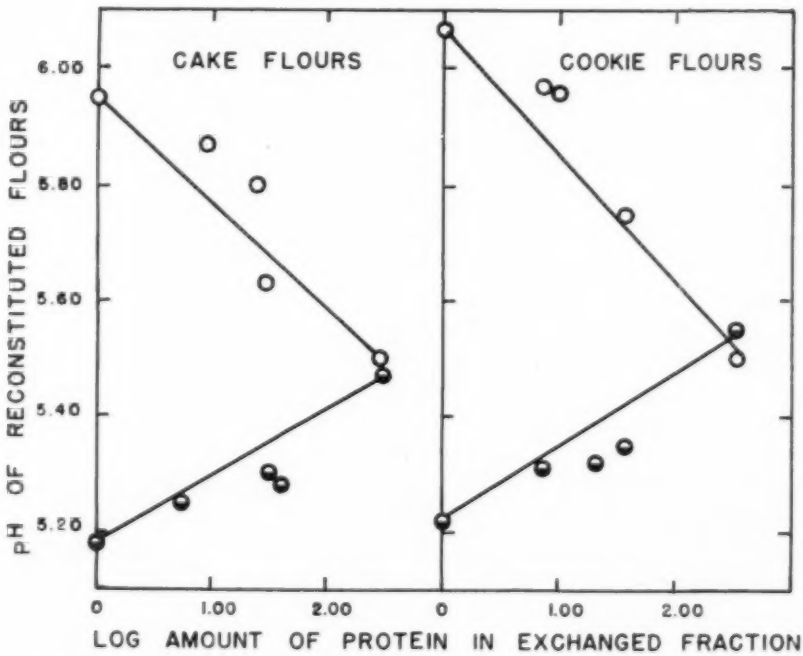

Fig. 1. Effect of the protein of the interchanged fraction on the pH of the reconstituted flours: ○, either a flour from four unbleached fractions or a flour from one bleached and three unbleached fractions; ◒, either a flour from four bleached fractions or a flour from one unbleached and three bleached fractions.

ed. Again, major responses were shown by gluten and prime starch, with water-solubles and tailings giving occasional small, sometimes contradictory, responses.

If the chlorination of starch increases hydration capacity, as maintained by Alexander (1), then the effect of bleached prime starch on cookie diameter is understandable. Yamazaki (11) has shown that there is an inverse relationship between water-holding capacity of a flour and cookie diameter.

Changes in pH Caused by Interchange of Fractions. In acid fractionations, the pH of fractions was adjusted to arbitrary levels, about 6.0 for those from unbleached flour and about 5.0 for those from bleached flour. The water-soluble fraction from unbleached flour was obtained without pH adjustment.

The effect of each fraction on the pH of the reconstituted flour was determined (Table III). Of the total change in pH, the largest portion, nearly 50%, was caused by gluten. The smallest portion, 8 to 15%, was caused by prime starch. Reconstituted cookie flours showed a similar behavior pattern. This effect on pH was roughly proportional to the amount of protein in the interchanged fraction. This relationship is shown in Fig. 1 where the logarithm of the amount of protein in the interchanged fraction is plotted against pH for that reconstituted flour. The changes in pH must be almost entirely due to the protein of bleached flour.

Conclusions

Chlorine bleaching affects the gluten and prime starch fractions of cake and cookie flours, causing changes in baking quality. The effect on other fractions is too small to be important. Unbleached prime starch is the fraction responsible for the collapse of yellow layer cakes from unbleached flour.

Acknowledgments

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DIFFERENTIAL EFFECT OF DILUTE ALKALI ON 25 VARIETIES OF MILLED WHITE RICE[1]

RUBY R. LITTLE, GRACE B. HILDER, AND ELSIE H. DAWSON[2]

ABSTRACT

The dispersal of material from white rice kernels upon treatment for 23 hours with 1.7% potassium hydroxide was evaluated for spreading and clearing on 7-point numerical scales. Sixty-five lots of rice milled from 25 varieties, representing three crop years and four geographical areas of the United States, were included in the study. Slight-to-moderate spreading and clearing was characteristic of most long-grain varieties, and a more pronounced reaction was charactertistic of most medium- and short-grain varieties. Exceptions were noted, and also the possible influence of growing conditions and storage time. Values for spreading and for clearing were negatively correlated with panel scores for cohesiveness at the 1% level of significance, with coefficients of −0.51 and −0.85, respectively.

Microscopic observations of the dispersed material revealed that it consisted of starch in the form of granules and in nongranular form. Starch granules were of two types: (a) "swollen" granules or those having a dilated appearance and often indefinite outline, and (b) "compact" granules or those which seemed to have lost in density without expanding. Nongranular starch could be seen in patterns roughly classified as very fine, fine, medium, and coarse.

Warth and Darabsett (4) examined eight Asiatic short- and medium-grain varieties after treatment in several concentrations of potassium hydroxide. Two main types of rice were noted, less-resistant and resistant, corresponding to differences in hardness, milkiness, and milling qualities of the different rices. A relationship to the "quality of starch" was postulated on the basis of comparison with gelatinization temperatures for three varieties.

Jones (2) soaked milled kernels in a 2.38% solution of potassium hydroxide for 24 hours and noted the resulting patterns of disintegration. Roughly three types of rice could be distinguished: kernels disintegrating into opaque masses, kernels disintegrating into clear masses, and kernels which were intermediate. Those varieties disintegrating into clear or intermediate masses were generally considered to have preferable cooking quality. Interpretation of the results was largely subjective, both as to classification of borderline cases and as to meaning in terms of cooking characteristics in the absence of evaluations by taste panels. Variations attributed to location and conditions of growth and to conditions prevailing during the experiments were noted.

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TABLE I

MEAN VALUES FOR SPREADING AND CLEARING OF 25 VARIETIES OF MILLED WHITE RICE TREATED FOR 23 HOURS IN 1.7% POTASSIUM HYDROXIDE

VARIETY	LOT NO.	SOURCE	CROP YEAR	SPREADING[a]		CLEARING[b]	
				March 1956	Sept. 1956	March 1956	Sept. 1956
LONG-GRAIN							
Bluebonnet	1	Tex.	1954	2.9	2.7	2.1	1.7
Bluebonnet 50	2	Tex.	1954	4.0	3.0	2.6	2.0
	3	Tex.	1954	4.4	3.7	3.2	2.8
	4	Tex.	1954	4.7	5.0	3.5	4.2
	5	Tex.	1955	5.0	3.6	2.2	2.4
Century Patna 231	6	Tex.	1953	2.0	2.0	1.0	1.0
	7	Tex.	1953	2.0	2.0	1.0	1.0
	8	Tex.	1954	2.0	2.0	1.0	1.0
	9	Tex.	1954	2.0	2.0	1.0	1.0
	10	Tex.	1954	2.0	2.0	1.0	1.0
	11	Tex.	1955	2.0	2.1	1.0	1.1
Fortuna	12	Tex.	1954	2.8	3.0	2.2	1.9
	13	Tex.	1955	5.0	3.5	2.2	2.1
Improved Bluebonnet	14	Tex.	1954	4.4	3.0	2.0	2.0
	15	Tex.	1955	5.0	3.9	2.0	2.0
Rexark	16	Tex.	1954	6.0	6.2	6.2	4.0
Rexoro	17	Tex.	1953	4.0	5.0	3.6	4.0
	18	Tex.	1954	5.0	4.0	3.3	3.2
	19	Tex.	1954	4.9	3.0	2.0	2.2
	20	Tex.	1955	5.0	4.2	2.0	2.1
Sunbonnet	21	La.	1954	4.7	3.1	3.1	2.2
	22	Tex.	1954	4.3	3.0	2.6	2.1
	23	Tex.	1955	4.8	2.9	2.0	2.2
Texas Patna	24	Tex.	1954	4.7	5.0	3.2	3.7
	25	Tex.	1954	5.0	5.0	3.0	3.9
	26	Tex.	1955	5.0	3.9	2.0	2.4
Toro	27	La.	1954	6.0	7.0	6.8	5.9
	28	La.	1954	6.0	7.0	6.8	5.7
	29	Tex.	1955	6.0	7.0	6.8	5.6
TP 49	30	Tex.	1954	5.3	4.3	4.7	3.7
	31	Tex.	1955	5.0	4.4	2.6	2.6
B4512A1-20	32	Tex.	1954	3.2	2.9	2.2	2.0
B4512A1-32 (hybrid)	33	Tex.	1955	5.0	2.9	2.0	1.9
B455A1-25 (hybrid)	34	Tex.	1954	4.3	2.9	1.8	2.0
	35	Tex.	1955	5.0	2.9	2.1	2.0
MEDIUM-GRAIN							
Blue Rose	37	Tex.	1954	6.0	6.0	5.8	4.7
	38	Tex.	1955	6.0	6.6	6.6	5.8
Calrose	39	Tex.	1954	6.0	6.0	5.8	5.0
	40	Calif.	1954	6.3	7.0	7.0	6.3
	41	Tex.	1955	6.0	5.6	5.9	4.5
Early Prolific	42	Tex.	1954	2.0	2.0	1.0	1.0
	43	Tex.	1955	2.7	2.2	1.7	1.2
Magnolia	44	Tex.	1954	5.8	6.0	5.4	4.8
	45	Tex.	1955	6.0	6.0	5.0	4.0
Nato	46	Tex.	1954	6.0	7.0	6.0	5.9
	47	Tex.	1955	6.0	6.7	6.0	5.3
Zenith	48	Tex.	1953	5.9	6.0	6.1	5.6
	49	Tex.	1953	6.0	6.0	6.2	5.1
	50	Tex.	1954	6.0	7.0	6.6	5.8
	51	Ark.	1954	5.7	7.0	6.4	6.8
	52	Tex.	1955	5.0	5.8	6.6	5.0

(Continued)

TABLE I (Continued)

Variety	Lot No.	Source	Crop Year	Spreading[a]		Clearing[b]	
				March 1956	Sept. 1956	March 1956	Sept. 1956
Short-Grain							
"California Pearl"	53	Calif.	1954	6.5	7.0	7.0	7.0
Caloro	54	Tex.	1953	6.0	6.0	6.0	4.0
	55	Tex.	1953	6.0	6.0	6.2	4.0
	56	Tex.	1954	6.0	6.0	6.0	4.9
	57	Calif.	1954	7.0	7.0	7.0	7.0
	58	Calif.	1955	6.0	7.0	7.0	7.0
	59	Tex.	1955	6.0	6.0	6.0	4.8
Colusa	60	Tex.	1954	6.0	6.0	6.0	5.0
	61	Calif.	1954	6.5	7.0	7.0	7.0
	62	Tex.	1955	6.0	5.8	5.7	5.0
11-47-11-1 (hybrid)	63	Tex.	1954	6.0	7.0	6.7	5.9
	64	Tex.	1955	6.0	7.0	6.9	6.9
12-47-6-2 (hybrid)	65	Tex.	1954	6.0	5.0	6.0	5.0
	66	Tex.	1955	6.0	7.0	6.0	5.0

[a] Spreading scale: 1, kernel not affected; 2, kernel swollen; 3, kernel swollen, collar incomplete or narrow; 4, kernel swollen, collar complete and wide; 5, kernel split or segmented, collar complete and wide; 6, kernel dispersed, merging with collar; 7, all kernels completely dispersed and intermingled.

[b] Clearing scale: 1, kernel chalky; 2, kernel chalky, collar powdery; 3, kernel chalky, collar cottony or cloudy; 4, center cottony, collar cloudy; 5, center cottony, collar clearing; 6, center cloudy, collar cleared; 7, center and collar cleared.

The present study was made to ascertain the reactions to dilute alkali of present varieties of rice; to determine whether or not results from the alkali treatment, applied to small samples, could be correlated with results from evaluations of larger samples by a taste panel; to improve control of conditions; and to develop an objective scale for measuring differences in kernel disintegration. Since preliminary trials gave hopeful results, in 1956 and 1957, a modification of previously published procedures was applied to 65 lots of rice being subjected to several other tests and examinations including panel evaluations (1).

Materials and Methods

Materials. The 65 lots of rice used in this study are listed in Table I according to grain type, variety, source, and crop year. Harvesting, drying, and milling were done by conventional methods at other locations prior to submission of the samples to this laboratory in 1954 and 1955. All lots of rice were stored at 38°–40°F. (3.3°–4.4°C.) from the time of receipt until required for experiment. Each lot was tested in March 1956. The test was repeated on all lots after they had been stored an additional 6 months at 38°–40°F. (3.3°–4.4°C.), and on a few lots from the 1955 crop after 12 months (March 1957). Samples for miscroscopic observations were prepared during the interval between March and September 1956, and samples for photography were prepared in August 1956.

In preliminary trials, kernels of Century Patna 231 (lot 10) and Caloro (lot 57), respectively showed the least and the greatest reaction to dilute potassium hydroxide solution. The concentration adopted for routine testing was chosen as a result of soaking kernels from these two samples in nine dilutions, namely; 1.66, 1.71, 1.74, 1.76, 1.80, 1.85, 1.96, 2.01, and 2.06%.[3] The Caloro kernels disintegrated into clear or slightly cloudy masses in all dilutions, whereas the Century Patna 231 sample was merely swollen when soaked in 1.66 to 1.76% potassium hydroxide, very slightly dispersed in 1.80 and 1.85%, and partially disintegrated into opaque masses in the higher concentrations. Since soaking in 1.7±0.05% potassium hydroxide solution gave results at the lower and upper ends of the possible range, this solution was selected as the best for our purposes.[4]

Procedure. The tests were performed in an air-conditioned room at 70°–75°F. (21°–24°C.). Rice samples were held at this temperature for 1 to 1½ hours before being immersed in the reagent. Relative humidity of the air varied from 30 to 60% depending upon the season, but since the containers were tightly covered this variation does not appear to have affected the results. Six rice kernels comprising a sample were spaced evenly in a small transparent plastic box, size 1⅞ by 1⅞ by ¾ in.,[5] containing 10 ml. of the reagent (1.7% potassium hydoxide solution). The cover was put on and the box, which was on a black surface, was left undisturbed during the test period. Final observations were made at the end of 23 hours, since no appreciable change occurred afterwards, and the next group could then be started at the same time of day. Century Patna 231 rice, lot 10, was the control; dispersion of the control was taken as evidence of unusual conditions and the results of such replications were discarded. Three replications of the treatment were made on each lot of rice, with added replications for lots showing unusual variation.

Detailed notes were made on the condition of samples, kernel by kernel in case of variation within the sample. The alterations were described as two separate although parallel reactions, namely, extent of dispersion or spreading, and degree of clearing. Seven-point scales, comparable to the score sheets used in panel evaluations, were devised for estimating the extent of those two reactions separately (Table I, footnotes) and each kernel was given a numerical value or score depending on its position on the scale. These values were averaged by lot and by variety and compared with panel scores for cohesiveness.

For microscopic observations, single kernels were soaked in 2 ml.

[3] Concentration percentages were checked by titration.

[4] At the beginning of each series of tests, a stock solution containing approximately 4% potassium hydroxide (wt/vol) was made, and 250 ml. of approximately 1.7% solution prepared from this as needed.

[5] Obtained from R. P. Cargille Laboratories, Inc., 117 Liberty Street, New York 6, N.Y.

of 1.7% potassium hydroxide solution in small transparent plastic boxes (¾ by ¾ by ¾ in.[5]), in three or more replications. After 23 hours, about 0.25 ml. of the fluid surrounding the kernel was withdrawn from the sample with a fine pipet, so as to secure dispersed material but not to disturb the undispersed portion of the kernel. The fluid was released on a clean slide, and spread the length of a coverglass by one movement to the left and one to the right with the side of the pipet. After thorough drying on a histological warming table (about 38°C.), five slides at a time were collodionized, immersed 10 minutes in 95% alcohol, immersed 60 minutes in a Coplin jar of freshly prepared 70% alcohol containing 1% hydrochloric acid, and stained in an iodine chamber (3). Observations were made with white light and with polarized light, under 16-mm. and 1-mm. (oil immersion) lenses.

Results

Gross Observations. Figures 1–6 show the appearance of 22 samples, each a different variety, before and after treatment for 23 hours in 1.7% potassium hydroxide solution. Mean values for spreading and for clearing of all lots tested in March and September 1956 are shown in Table I. In Fig. 7 is shown the percent distribution of tested samples on the spreading and clearing scales during the September 1956 tests.

Long-grain rices were most variable in reaction to the alkali test, both within and among varieties. Century Patna 231 samples were scored consistently, 2 for spreading and 1 for clearing, although a slight tendency to spread was observed in later trials in March 1957. Except for a few individual kernels the other long-grain varieties underwent more spreading and more clearing, with all Toro and most Rexark kernels scored 5 to 7. Samples which had been grown in Arkansas or Louisiana did not differ from those grown in Texas. Broken kernels of the Rexoro and Century Patna 231 varieties behaved essentially like whole kernels of the same varieties.

Medium-grain varieties usually received values of 6 and 7 for spreading and 5 to 7 for clearing. Early Prolific samples, however, received values of 2 and 3 for spreading (the kernels being merely swollen, some with a small, fan-shaped partial collar) and 1 or 2 for clearing. Thus, this variety usually behaved like Century Patna 231 of the long-grain rices. Calrose rice grown in California cleared and spread more than that grown in Texas.

The short-grain varieties were usually given values of 6 and 7 for spreading, and 5 to 7 for clearing. California-grown samples of the Caloro and Colusa varieties usually underwent more spreading and more clearing than Texas-grown samples of the same varieties.

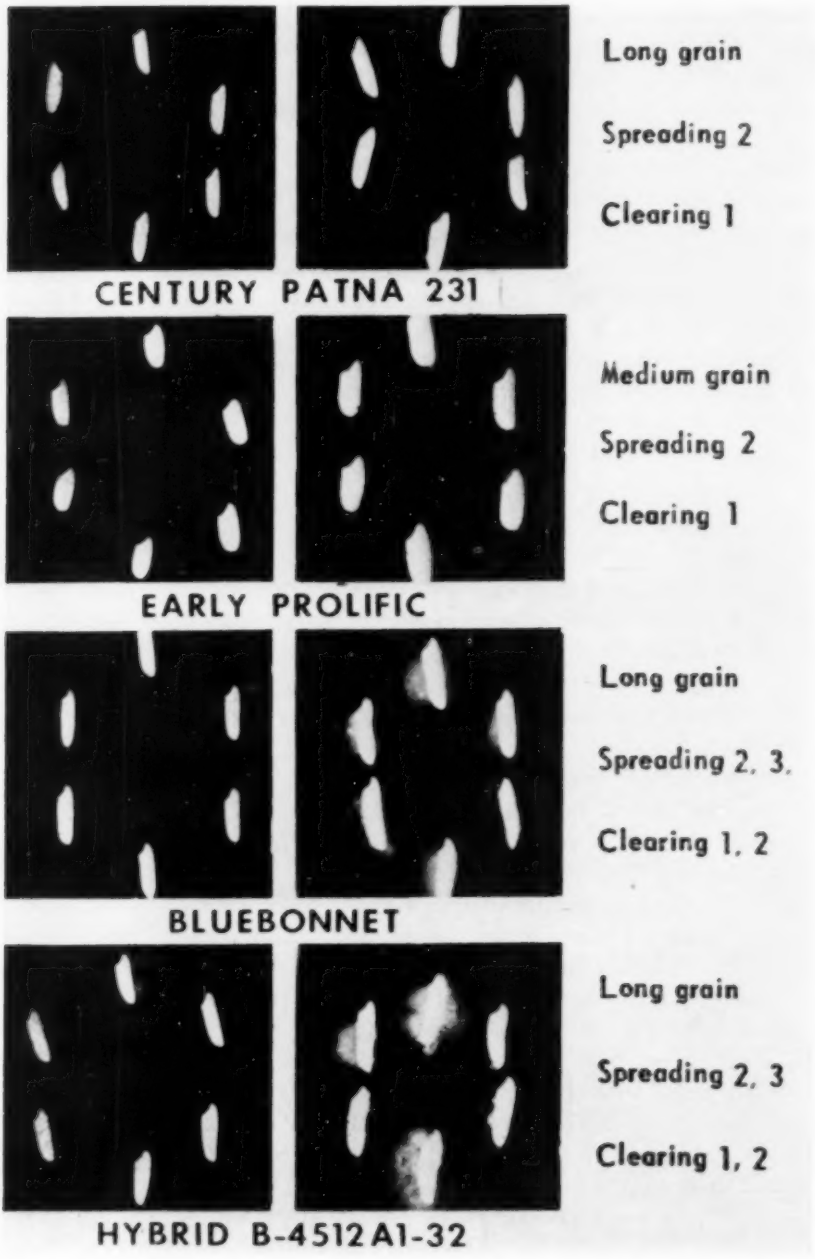

Fig. 1. Milled white rice of different varieties before (left) and after (right) treatment for 23 hours in 1.7% potassium hydroxide solution, illustrating spreading values of 2 to 3 and clearing values of 1 to 2. (Description of rating scales given in Table I, footnotes.

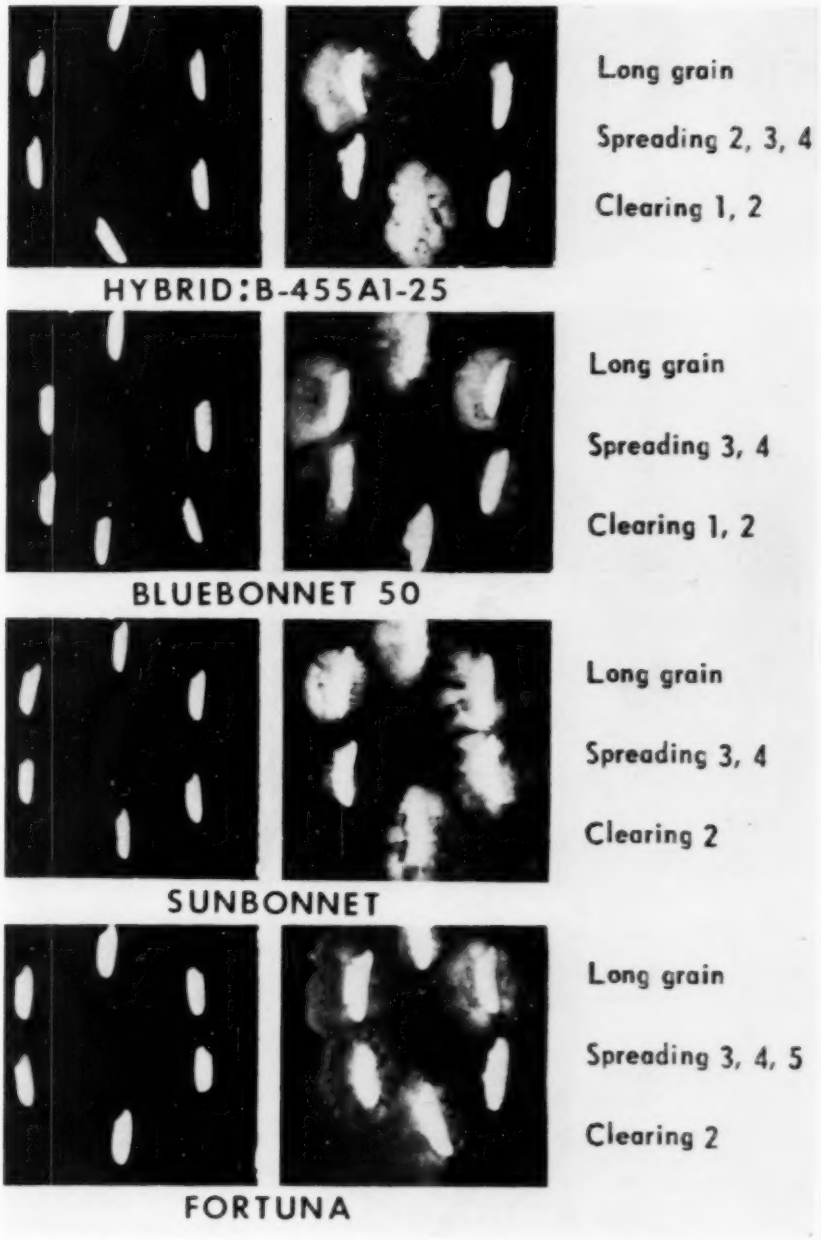

Fig. 2. Milled white rice of different varieties before (left) and after (right) treatment for 23 hours in 1.7% potassium hydroxide solution, illustrating spreading values of 2 to 5 and clearing values of 1 to 2. (Description of rating scales given in Table I, footnotes.)

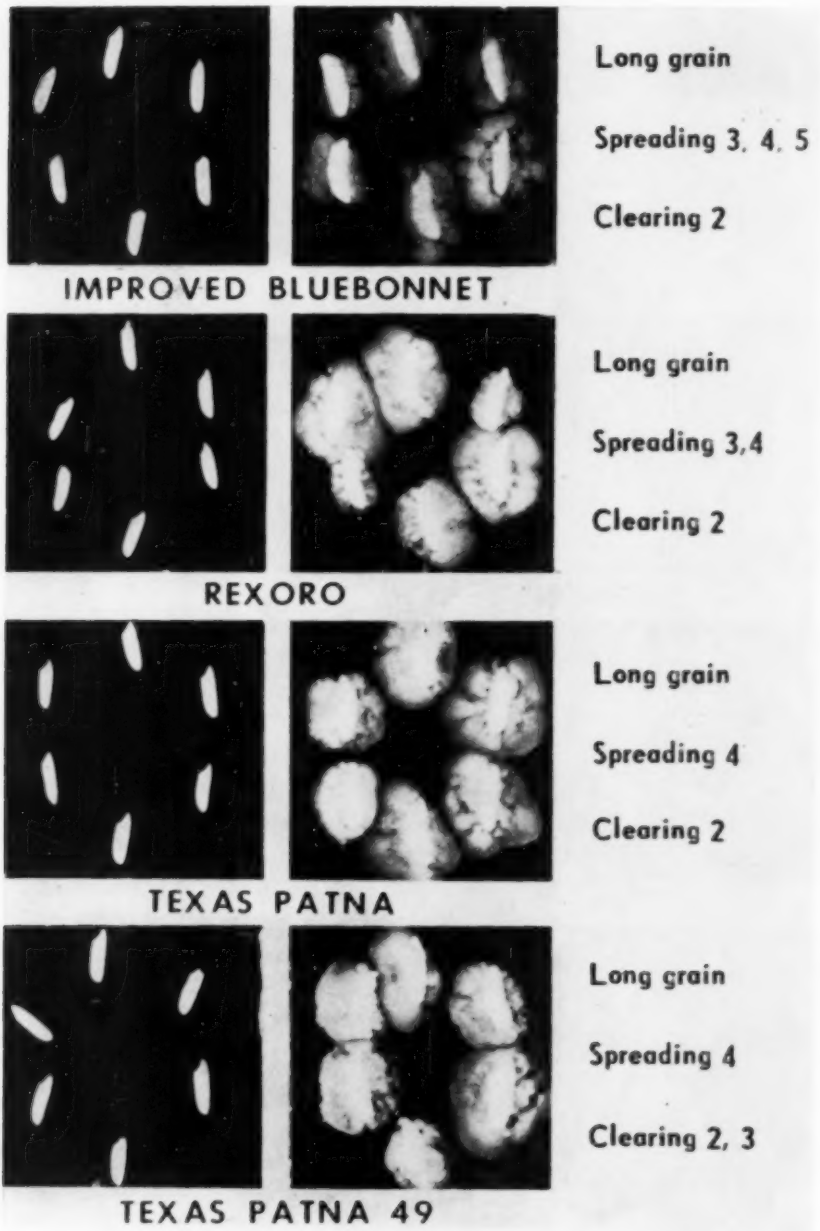

Fig. 3. Milled white rice of different varieties before (left) and after (right) treatment for 23 hours in 1.7% potassium hydroxide solution, illustrating spreading values of 3 to 5 and clearing values of 2 to 3. (Description of rating scales given in Table I, footnotes.)

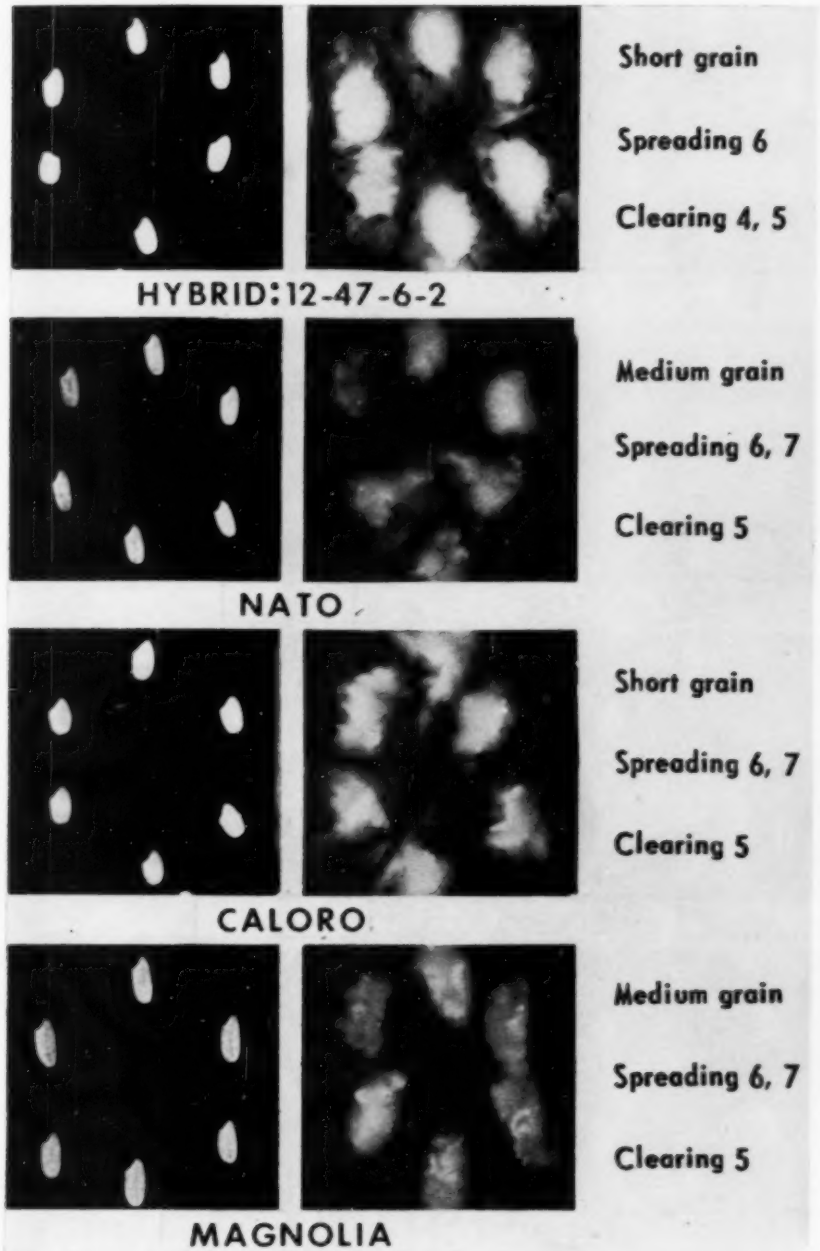

Fig. 4. Milled white rice of different varieties before (left) and after (right) treatment for 23 hours in 1.7% potassium hydroxide solution, illustrating spreading values of 6 to 7 and clearing values of 4 to 5. (Description of rating scales given in Table I. footnotes.)

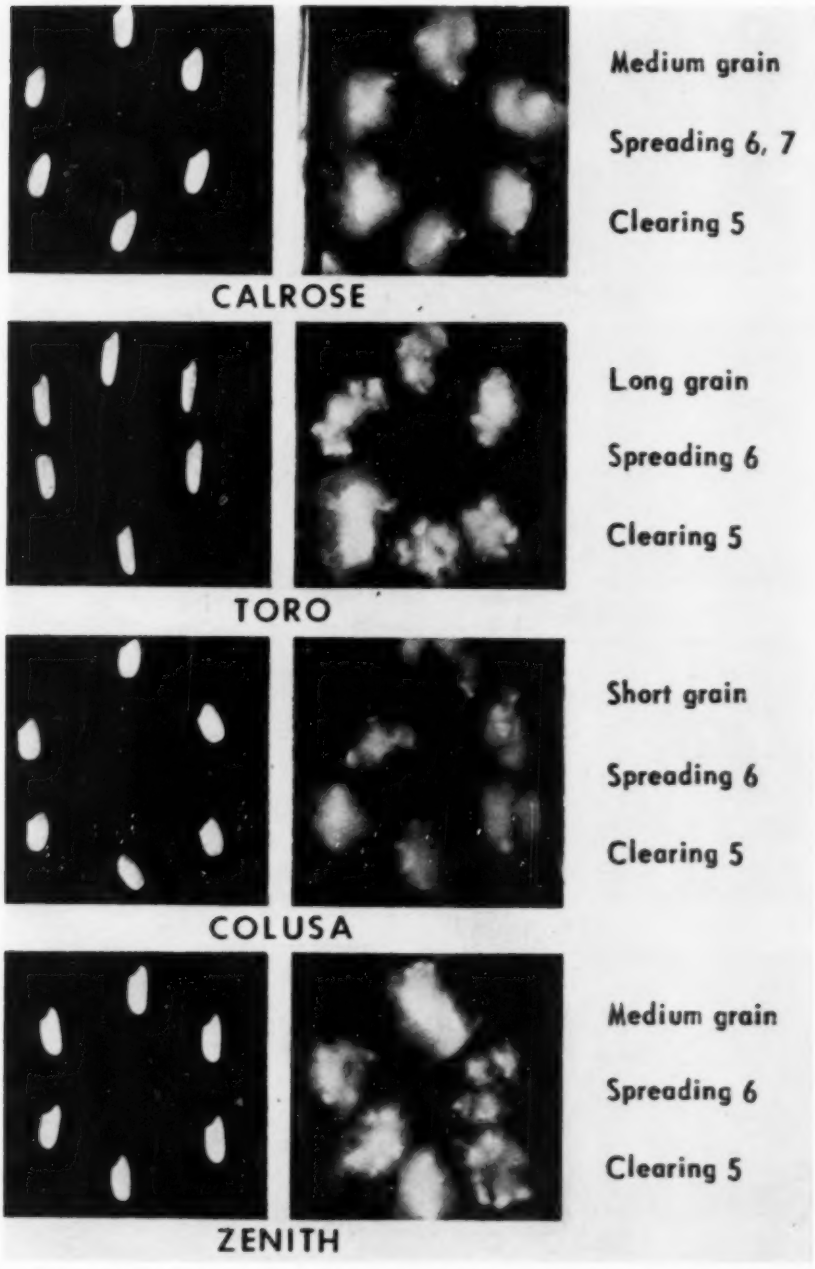

Fig. 5. Milled white rice of different varieties before (left) and after (right) treatment for 23 hours in 1.7% potassium hydroxide solution, illustrating spreading values of 6 to 7 and clearing value of 5. (Description of rating scales given in Table I, footnotes.)

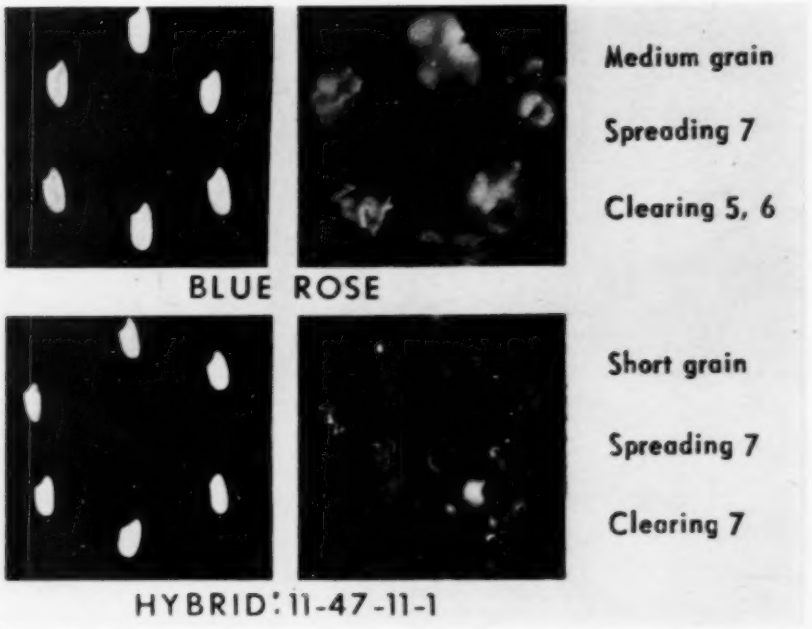

Fig. 6. Milled white rice of different varieties before (left) and after (right) treatment for 23 hours in 1.7% potassium hydroxide solution, illustrating spreading value of 7 and clearing values of 5, 6, and 7. (Description of rating scales given in Table I, footnotes.)

In several varieties, a decrease or increase in spreading or clearing with additional storage was noticed. Several of the long-grain varieties had lower values for spreading after 6 months of storage at 38°–40°F. (3.3°–4.4°C.), whereas some lots of the medium- and short-grain varieties had higher values for spreading and lower values for clearing after storage. These reactions may be indicative of chemical or physical changes in milled white rice during holding periods.

Microscopic Observations. Microscopic observations revealed the presence of two types of starch granules, (a) "swollen" and (b) "compact"; starch was present also in nongranular form (c). Starch granules of the "swollen" type looked as though they might have reacted to the alkali treatment by swelling, becoming less dense, and finally spreading out to form a circular, homogeneous or finely particulate, lightly staining mass of indefinite outline. In polarized light these granules usually were weakly birefringent about the circumference, but they sometimes showed no birefringence and conversely sometimes their outlines were impossible to make out in white light. They were present in the fluid surrounding kernels showing little or no spreading; this may indicate that they are a morphological type of starch granule occurring in the outer part of the rice endosperm.

Granules of the compact type (Fig. 8, A) had much the same appearance as unaltered rice starch granules in size, shape, and pattern of

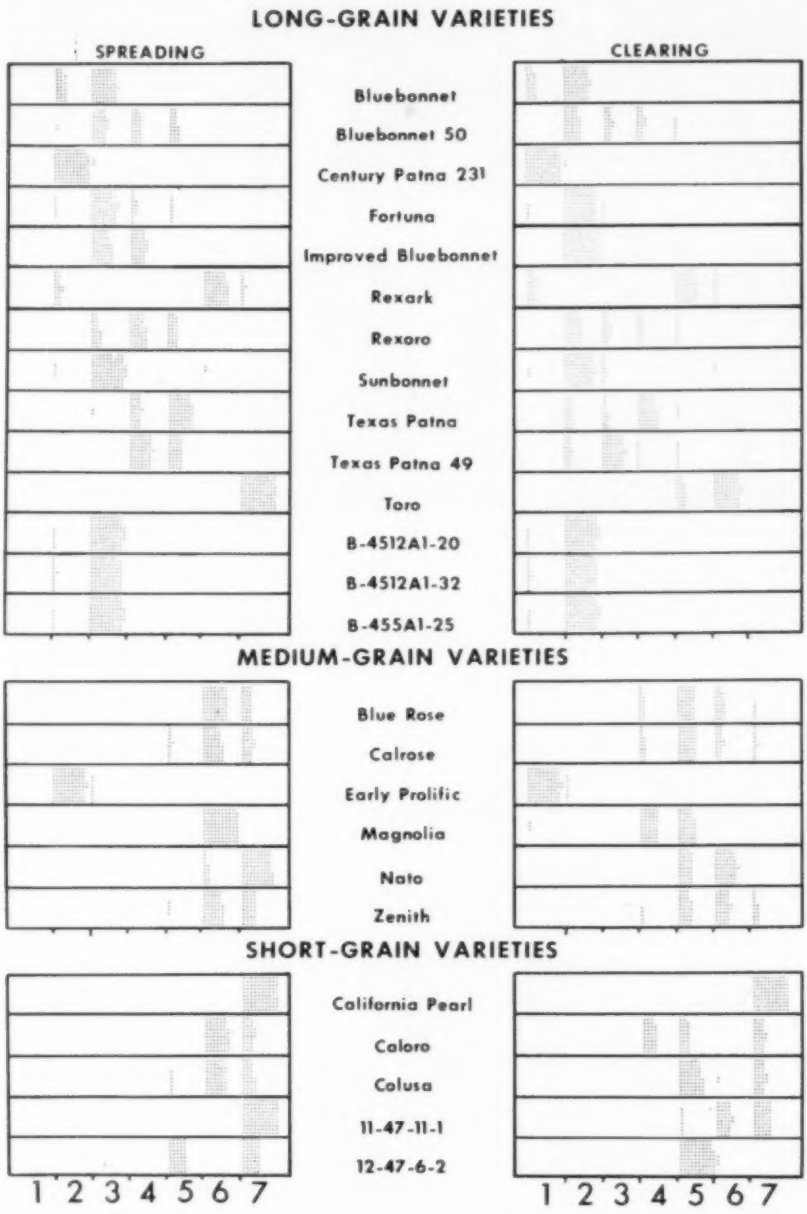

Fig. 7. Percent distribution of rice varieties on spreading and clearing scales, September 1956. (Spreading and clearing scales given in Table I, footnotes.)

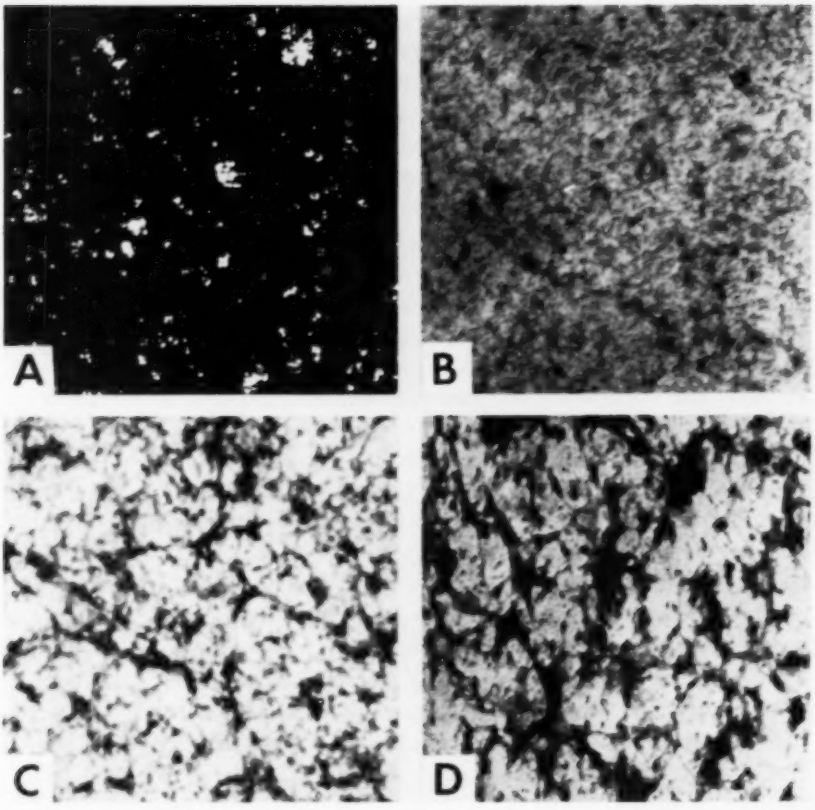

Fig. 8. Photomicrographs showing differences in rice starch dispersed from kernels soaked in 1.7% potassium hydroxide. A, compact-type granules as seen in polarized light; B, C, D, nongranular starch as seen in white light; B, fine formation; C, medium formation; D, coarse formation. Magnification 200X.

birefringence. They showed almost no color from the iodine stain and in many cases escaped detection in white light; often they could be located only by the presence of a hilum or central globule. Their birefringence was normal or weakened, not confined to the periphery of the granules as in the "swollen" type. Sometimes they appeared to have been halved or to have lost a "quarter." A range of sizes could be discerned in many slides, probably a reflection of the range originally present.

Nongranular or dissolved starch, when dried on the slide and stained with iodine (Fig. 8, B, C, D) took the form of blue-to-purple, homogeneous, thinly spread masses, or fine-to-coarse particles, lumps, fibers, strands, films, or sheets, discrete or joined in a network. The patterns assumed by the nongranular starch may be roughly classified as very fine, fine, medium, and coarse.

In Table II, the varieties are tabulated according to 1) presence or absence of "swollen" granules, "compact" granules, and nongranular starch, and 2) coarseness or fineness of nongranular starch. Many slides showed more than one coarseness pattern, in which case they were placed according to the coarsest starch present. The results seemed to parallel those from gross observations, although fewer samples were used. Powdery collars probably consisted largely of granules with little nongranular or dissolved starch. Cottony or cloudy collars probably consisted of granules and nongranular starch. Clear or transparent samples probably consisted mostly or entirely of nongranular or dissolved starch.

Among the long-grain rices, Century Patna 231 differed from all others in the occurrence of swollen granules only, and in the absence of nongranular starch. Most of the slides made from Rexark and Toro rice fell at the opposite end of the scales, showing absence of granules and presence of medium-to-coarse lumps, strands, and sheets of nongranular starch. The other long-grain varieties were generally intermediate, having swollen granules, compact granules (either or both), and fine-to-medium particles, strands, and films of nongranular starch.

Among the medium-grain rices, Early Prolific was the only variety showing no nongranular starch. Most slides from the other medium-grain varieties and all from the short-grain rices showed absence of swollen granules, absence or presence of compact granules, and presence of medium-to-coarse nongranular starch. Slides made from California-grown samples of Calrose, Caloro, and Colusa rice had fewer granules (usually none) and more coarsely patterned nongranular starch than the Texas-grown samples.

Correlation with Other Quality Measurements. With 1955 samples, correlation coefficients were calculated for spreading and for clearing values compared with panel scores for cohesiveness or tendency of cooked rice kernels to stick to each other. A high score for cohesiveness of cooked rice indicated well-separated kernels that did not clump (1). The correlation coefficient for spreading and cohesiveness was −0.51, barely significant at the 1% level; and that for clearing and cohesiveness was −0.85, significant at the 1% level. The latter correlation, which is high enough to show some promise, signifies that low values for clearing are associated with high scores for lack of cohesiveness or nonsticky cooked rice. Significant correlation with other measurements of rice quality was not demonstrated.

Discussion

Modifications of previously published methods for preliminary

TABLE II

DISTRIBUTION OF 25 VARIETIES OF MILLED WHITE RICE ACCORDING TO MICROSCOPIC CHARACTERISTICS OF STARCH DISPERSED FROM RICE KERNELS IN 1.7% POTASSIUM HYDROXIDE

VARIETY	No. OF LOTS	REPLICATIONS (ONE KERNEL EACH)	TYPES OF STARCH ON SLIDES							CHARACTERISTICS OF NONGRANULAR STARCH				
			Nongranular Starch Absent			Nongranular Starch Present				No Nongranular Starch on Slide	Nongranular Starch Present			
			Swollen Granules Present		Swollen Granules Absent,	Swollen Granules Present		Swollen Granules Absent			Formation Very Fine	Formation Fine	Formation Medium	Formation Coarse
			Compact Granules Absent	Compact Granules Present	Compact Granules Present	Compact Granules Present	Compact Granules Absent	Compact Granules Present	Compact Granules Absent					
			(Type a)	(Type ab)	(Type b)	(Type abc)	(Type ac)	(Type bc)	(Type c)					
LONG-GRAIN														
Bluebonnet	1	3	..	..	..	2	1	..	..	..	..	3	..	..
Bluebonnet 50	4	15	..	..	..	9	3	2	1	..	1	9	4	1
Century Patna 231	5	15	13	..	2	..	..	..	..	15	..	..	..	..
Fortuna	2	6	1	..	..	3	2	..	..	1	..	5	..	..
Improved Bluebonnet	2	9	..	..	..	7	2	..	..	..	3	2	4	..
Rexark	1	4	..	..	..	..	..	1	3	..	..	1	..	3
Rexoro	4	20	..	..	..	14	..	5	1	..	..	17	3	..
Sunbonnet	3	12	..	..	..	10	..	2	..	..	..	5	6	1
Texas Patna	3	11	..	..	..	9	1	1	..	..	..	6	5	..
Texas Patna 49	2	7	..	..	..	2	1	4	..	..	..	6	1	..
Toro	3	12	..	..	..	..	1	3	8	..	..	5	3	4
B4512A1-20	1	4	..	3	..	1	..	..	..	3	1	..	..	..
B4512A1-32	1	3	..	..	..	2	1	..	..	..	..	3	..	..
B455A1-25	2	7	..	..	..	5	..	2	..	..	2	5	..	..
MEDIUM-GRAIN														
Blue Rose	2	6	..	..	..	..	..	3	3	..	..	..	..	6
Calrose:														
(Texas)	2	5	..	..	..	..	..	4	1	..	..	3	2	..
(Calif.)	1	3	..	..	..	..	..	..	3	..	..	..	..	3
Early Prolific	2	6	2	3	1	..	..	..	..	6	..	..	..	..
Magnolia	2	7	..	..	..	..	..	5	2	..	..	..	4	3
Nato	2	6	..	..	..	..	4	..	2	..	..	1	..	5
Zenith	5	18	..	..	..	..	..	4	14	..	..	5	5	8
SHORT-GRAIN														
"Calif. Pearl"	1	3	..	..	..	..	..	..	3	..	..	..	2	1
Caloro (Texas)	4	13	..	..	..	..	1	12	..	..	2	3	2	6
(Calif.)	2	5	..	..	..	..	1	..	4	..	..	..	1	4
Colusa (Texas)	2	6	..	..	..	..	..	5	1	..	..	2	1	3
(Calif.)	1	3	..	..	..	..	..	..	3	..	..	..	1	2
11-47-11-1	2	7	..	..	..	1	..	3	3	..	..	..	2	5
12-47-6-2	2	8	..	..	..	..	..	6	2	..	..	2	3	3

classification of rice varieties by treatment with dilute alkali recommended as a result of this study are: 1) treatment of milled kernels with 1.7% ± 0.05% potassium hydroxide solution, which allows a wide range in reactions among different varieties of rice; 2) maintenance of a moderate, fairly even room temperature during the test period; and 3) use of descriptive 7-point rating scales for estimating degrees of spreading and clearing. A numerical rating scale enables the investigator to make a more objective analysis of the results. It is likely that when other varieties are tested, other patterns may be encountered, in which case the scales might need revision.

The small plastic boxes are convenient and inexpensive containers which hold the amount of fluid needed for a single sample. When each sample is treated separately, the scoring seems less difficult and photographs made for study can be sorted and rearranged by type as desired.

Evidence is presented that the response of milled white rice kernels to treatment with 1.7% potassium hydroxide is largely a varietal characteristic and may be used as an indication of the textural qualities likely to be found in palatability testing of the same rice. The correlation between the reactions to alkali and quality evaluations by a panel are promising but are not high enough to replace panel evaluations with the chemical test without confirmation. More research is needed both for a more critical appraisal of this relationship and for possible increased accuracy, objectivity, and basic understanding of the disintegration process. A continuing program of research encompassing the reactions of many other varieties, samples from other geographical areas, and samples stored at different temperatures for various lengths of time should further these objectives. Suitable tests are needed to clarify the influence of environmental conditions of growth and the effects of harvesting, milling, and storage conditions on the characteristics of rice of different varieties.

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COMPOSITION OF THE CEMENTING LAYER AND ADJACENT TISSUES AS RELATED TO GERM-ENDOSPERM SEPARATION IN CORN[1]

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ABSTRACT

Composition of the region at the germ-endosperm interface in the corn kernel was studied histochemically and by using enzymes as specific reagents to obtain information basic to the improvement of industrial degermination. The region is comprised of a compressed layer in the endosperm, the scutellar epithelium of the germ, and an amorphous cementing layer between the two.

Cellulose and pentosans were found in the compressed layer. The cementing layer contained protein and pentosans. The cell walls of the scutellar epithelium showed the presence of cellulose and pentosans. Pectic substances were absent in the compressed layer and probably absent in the cementing layer and scutellar epithelium.

Microscopic observations of sections treated with enzyme preparations showed that separation of germ and endosperm occurred most easily over the tips of the epithelial cells. Separation appeared to be associated particularly with pentosanase activity. Confirmation was obtained by examination of the enzymic hydrolysates by paper chromatography, which showed that pentosan degradation products predominated. An attack on the relatively labile pentosans, particularly those in the epithelial cell walls, appears to offer better possibilities for efficient degermination than does an attack on the more resistant components of the cementing and compressed layers.

Examination of the corn kernel immediately discloses a sharp discontinuity between germ and endosperm tissues. This discontinuity offers a natural line of cleavage along which the parts might be expected to separate during milling. In actual industrial practice, however, a clean separation of germ and endosperm is difficult to obtain.

Some idea of the efficiency of industrial degermination may be obtained from oil recovery. Corn contains about 5% oil, about 85% of which is in the germ. If degermination and oil separation were perfect, about 2.3 lb. of oil would be recovered from a bushel of corn. Actually, approximately 1.6 lb., or about 70% of the germ oil, are recovered in wet milling (3), and only 0.6 to 0.7 lb., or about 28% of the oil present in the germ, in dry milling (7). Degermination in wet milling is relatively good; most of the unrecovered oil is in the oil cake. In dry milling, however, nearly three-fourths of the oil is in the hominy feed; this indicates poor degermination. Poor degermination in dry milling is accompanied by low recovery of large-sized grits. Both low oil recovery and small grits are uneconomical. Fundamental infor-

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mation on which to base improved processes of degermination is an expressed need of the corn dry milling industry.

Previous study of the structure of the mature corn kernel (13, 14) showed a thin film of amorphous material lying between the germ

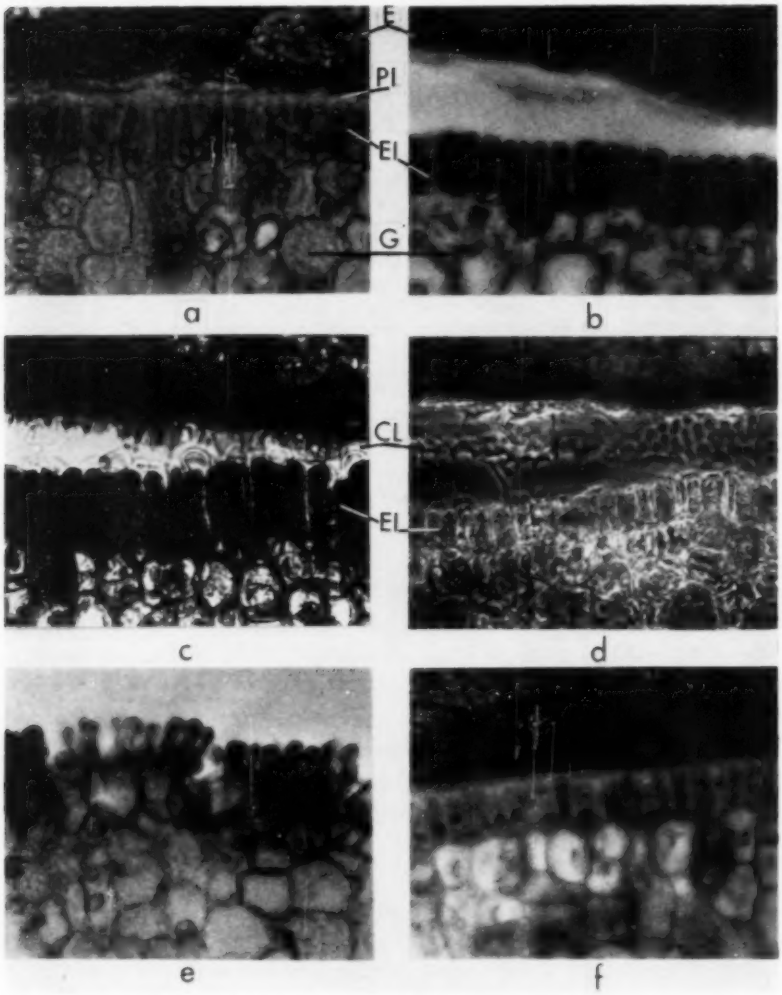

Fig. 1. Effect of incubation with enzymes on germ-endosperm separation and on staining characteristics of tissues at the germ-endosperm interface. All sections were stained with Congo red except d, which was photographed with phase contrast, without staining. a, Control, heat-inactivated Enzyme 19; b, Enzyme 19, 2 hours; c, Enzyme 19, 19 hours; d, cellulase-pentosanase fraction, 1 hour; e, cellulase-pentosanase fraction, 18 hours; f, pectic enzyme fraction, 29 hours; E, starchy endosperm; Pl, compressed layer of endosperm; CL, cementing layer; El, scutellar epithelium of germ; G, parenchyma cells of germ. (Magnification 169×.)

and endosperm. This material is now called the "cementing layer" because it appears to cement the two structures together. It was first reported by Sargant and Robertson (9), who called it a "dark line." They described it as a viscous, sticky substance of unknown composition and thought it to be a secretion of the epithelial cells of the scutellum. Directly adjacent to the cementing layer are endosperm cells largely devoid of contents and compressed into a narrow band. This is called the "compressed layer." On the germ side, the epithelial cells of the scutellum border directly on the cementing layer. These layers are shown in Fig. 1.

Results are reported here of the first phases of a study of the cementing layer, compressed layer, and scutellar epithelium. Separation along any of the layers in this interfacial region should result in high efficiency of industrial degermination.

Materials and Methods

Histochemical. Sections 30 or 40 μ thick were cut on the freezing microtome from kernels of U. S. 13 dent corn steeped overnight in distilled water at room temperature. All of the pericarp and much of the endosperm were removed prior to sectioning. Sections were washed repeatedly with distilled water, dehydrated by transfer through a series of alcohols to absolute ethanol, and defatted with several changes of petroleum ether (b.p. 33°–57°C.) during 8 to 10 hours. Fat solvent was removed with absolute ethanol and sections stored in 70% ethanol. The procedure removed most of the cell contents except from endosperm and scutellar epithelium.

The histochemical tests and reagents used were based on those of Tunmann and Rosenthaler (10). Differential staining and solubility tests, or color reactions and solubility tests, were combined to obtain specificity. For cellulose, coloration with iodine-potassium-iodide solution followed by sulfuric acid, coloration with chlor-zinc-iodide, and solubility in Schweitzer's reagent were employed. Coloration with a solution of Sudan IV in ethylene glycol (2) and relative insolubility in chromic acid were used as tests for cutin and suberin. Tests used for pectic substances included staining with Ruthenium red, extraction with hot 0.5% ammonium oxalate solution, and extraction with alternate heating in 2% hydrochloric acid and 2% potassium hydroxide solutions. For pentosans, the phloroglucinol color test, and extraction with hot 1% sulfuric acid solution were used. The Millon and xanthoproteic tests were used for proteins.

Enzymic. Sections for enzymic studies were prepared like those used for the histochemical tests.

Among a number of enzyme preparations tested, Pectinol W[3], Enzyme 19 (both obtained from Rohm and Haas Co., Philadelphia, Pa.), and a bacterial α-amylase (obtained from Wallerstein Laboratories, New York, N. Y.) effected a clean separation of germ from endosperm when applied to the sections. Enzyme 19, the most active of the group, was selected for further use. It was found to include α-amylase, protease, cellulase, pentosanase, polygalacturonase (PG), and pectinmethylesterase (PE) activities. Other activities, of no interest to the present problem, have been reported elsewhere.

Commercial Enzyme 19 was prepared for use by filtering off insoluble material and dialyzing against distilled water at 0°C. The enzyme was stored as a freeze-dried product.

Because the sections provided a mixed substrate, enzyme preparations containing one, or at the most two, activities were required to identify the component being degraded. A number of relatively simple systems were obtained by fractionation of Enzyme 19. The cellulase-pentosanase fractions contained little or no pectic enzyme activity. The pectic enzyme fractions contained both PG and PE activities and little or no cellulase and pentosanase activity. All of the fractions, except the cellulase prepared by heat treatment of Enzyme 19, showed some amylase and protease activities. These two activities were shown to be without effect on the interfacial area of the kernel. The cellulase preparation contained only cellulase activity. Details of the fractionation will be published elsewhere.

The effect of Enzyme 19 and of its fractions was studied by preparing a solution containing 2 mg/ml. in 0.1M, pH 4.5 acetate buffer with 0.01% Dowicide 4 (2-chloro-4-phenylphenol) as a preservative, and adding 60 to 200 sections per 5 ml. If the supply of material permitted, the concentration of the solutions of Enzyme 19 fractions was adjusted so that the total activity applied to the corn sections was about the same as that which gave the desired results in 1 to 2 hours when Enzyme 19 was used. The solution was then digested at 37° C. with shaking. A control was prepared with enzyme inactivated by heating at pH 4.5 at 94°C. for 15 to 20 minutes. Sample sections were removed at from 30 minutes to 58 hours. Microscopic examination and dissection showed the degree of separation of germ and endosperm; the staining characteristics of cell walls and cell contents were noted insofar as these might indicate degradation. A 0.1% solution of Congo red in pH 8.0 phosphate buffer was used for staining.

Chromatographic. Corn germ was excised to obtain relatively starch-

[3] The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

free material as a substrate. Sections were prepared as before. Residual starch, contained in a few fragments of endosperm adhering to the germ, was largely removed by treatment with malt alpha-amylase. Chiefly cell walls remained; the contents of scutellar epithelial cells and a little protein remaining in the endosperm were ignored. Digestion with enzyme was carried out, as described, for periods up to 24 hours. Samples in heat-inactivated enzyme solution and zero-time samples from the active enzyme were used as controls. The sections were filtered off after treatment and examined microscopically. Ethanol was added to the filtrate to a concentration of 80%. A gelatinous precipitate formed and was removed by centrifugation. The supernatant solution was evaporated to dryness at room temperature, the residue extracted twice with redistilled pyridine (6), and the pyridine evaporated off at room temperature. The residue was taken up in 0.2 ml. distilled water and spotted on No. 1 Whatman filter paper. The sheet was irrigated with an n-butanol, pyridine, water mixture, 3:2:1.5 (5). A mixture of ethyl acetate, pyridine, water, and acetic acid (5:5:3:1) (4) was used when the sections were treated with pectic enzyme. The sheets were sprayed with ammoniacal silver nitrate to detect sugars.

Photomicrographic. Sections stained with Congo red were mounted in 66% glycerol and photographed under the ordinary bright-field microscope. Some photomicrographs of the unstained, highly transparent cementing layer were obtained by use of the phase contrast microscope.

Results

The cementing layer was found to be optically isotropic; this was expected from its amorphous nature. All cell walls in the area were birefringent.

Composition from Histochemical Tests. The cementing layer gave a negative test for cellulose and dissolved slowly in the strong sulfuric acid of the hydrocellulose reagent. An absence of fats or suberized materials in the layer was indicated by its failure to stain with Sudan IV and by its relative ease of solubility in 50% chromic acid solution. Tests for proteins were inconclusive. (Recent preliminary ultramicro-Kjeldahl analysis of isolated material has shown the presence of considerable amounts of protein.) A positive test for pentosans was obtained. Pectic materials appeared to be absent, although the tests were not entirely conclusive.

The cell walls of the scutellar epithelium contained pentosans and cellulose, as indicated by both color and solubility tests. Pectic substances appeared to be absent, although a clear evaluation of the tests

was impossible. The epithelial cell walls were comparatively labile; relatively mild treatment with dilute acid or alkali caused their swelling or partial dispersion. Treatment with hot ammonium oxalate resulted in a marked increase in transparency of the walls; they became swollen when heating was prolonged.

Cellulose and pentosans, but no pectic substances, were found in the compressed layer.

Effect of Enzyme 19. Separation of germ and endosperm was obtained in the sections after about 1 hour of treatment with Enzyme 19 solution (Fig. 1). When pulled apart, germ and endosperm separated readily over the tips of the epithelial cells, without tearing of tissues. After longer periods of treatment, the sections separated along this line in the suspension, either spontaneously or on shaking (Fig. 1, b).

The compressed layer was relatively resistant to enzymic degradation. Separation through the compressed layer occurred in only a few trials and then only in some sections of any one sample. The rest separated preferentially over the tips of the epithelial cells.

The cementing layer survived the enzyme treatment. Its characteristic appearance when pulled away from the germ was that of a series of projections and depressions coinciding in size and shape with the tips of the epithelial cells (Fig. 1, c and d). Ordinarily, the projections were short; in some instances, however, they were long and thin. The projections appeared to represent cementing layer which originally filled in between the free ends of the epithelial cells.

In sections treated with Enzyme 19 for 20 hours or longer, cell walls were appreciably thinner and the sections were fragile. The tips of the epithelial cell walls were frequently missing, leaving only the basal portion attached to the scutellar parenchyma cells. Cell contents were often washed out from the cell remnants. A separation of individual cells along the middle lamella, such as is brought about by the action of pectic enzymes (maceration), was not observed. The fragility of the tissues resulted, rather, from degradation of the cell wall itself, with a consequent decrease in its tensile strength.

After 30 minutes or more of enzyme treatment (depending on the activity of the particular preparation), the sections showed a markedly greater affinity for Congo red than did the controls (see Fig. 1, a and b, c, e). The effect was particularly striking in the epithelial cells, most of which remained unopened by sectioning. Both the cell walls and the granular protein in the cell contents of the treated sections stained deeply and quickly with Congo red. In contrast, the contents of scutellar epithelial cells of untreated or control sections were at first unstained or lightly stained and required from several hours to overnight

in the staining solution to reach an appreciable depth of color. The changes in staining characteristics are regarded as evidence of partial enzymic degradation of the cell walls with consequent increased permeability to Congo red. This change in staining behavior always accompanied easy separation of germ and endosperm after enzyme treatment.

Similar increase in depth of staining with Congo red was noted after treatment of cotton fibers with cell-free enzyme solution from a cellulolytic fungus (1).

Effect of Fractions of Enzyme 19. The first problem was to determine which components of Enzyme 19 were acting on the cell walls of the scutellar epithelium. Optimum separation of germ and endosperm and accompanying changes in staining with Congo red occurred at pH 4.5. This point coincided with the pH optimum on their respective substrates of the pentosanase, cellulase, and pectic enzymes of the Enzyme 19 mixture. The protease component showed very little activity at pH 4.5 and had an optimum at pH 7.0. No separation of the germ and endosperm could be obtained at pH 7.0; consequently, protease was ruled out as contributing to the separation. The amylase component had an optimum at pH 5.0, but purified malt alpha-amylase applied to corn sections had no influence on the separation of germ and endosperm.

When the fractions were applied to corn sections, only the mixtures of cellulase and pentosanase showed the reactions typical of Enzyme 19 (Table I; Fig. 1, d and e). Activity on the insoluble substrate provided by the corn sections roughly paralleled that on the soluble substrates used for routine assay.

TABLE I

EFFECT OF VARIOUS ENZYME PREPARATIONS ON DEFATTED CORN SECTIONS

ENZYME OR FRACTION	PREPARATION	CONCENTRATION	SEPARATION OF GERM AND ENDOSPERM
		mg/ml.	
Enzyme 19		2.0	Complete in 1 to 2 hours
Cellulase and pentosanase[a]	A	2.0	Complete in 0.5 to 1 hour
	B	2.0	Complete in 3 hours
	C	2.5	Complete in 6 hours
	D	2.0	Complete in 5 hours
Pectic enzymes[a]	A	2.5	None in 9 hours
	B	2.0	None in 28 hours
	C	2.0	None in 29 hours
Cellulase[b]		4.0	None in 58 hours
Polygalacturonase (PG)[c]		2.0	None in 9 hours
PG + cellulase		2 + 10	None in 21 hours

[a] Preparations A, B, and C obtained by preparative paper chromatography of Enzyme 19; preparation D obtained by paper electrophoresis.
[b] Obtained by heat-treatment of Enzyme 19.
[c] Purified PG of fungal origin.

Fractions containing pectic enzymes and the preparations containing only cellulase activity were without effect on the germ-endosperm separation and staining characteristics of the sections, even after long periods of digestion (Table I; Fig. 1, f).

To summarize, enzymic degradation of the cell walls of the scutellar epithelium by a mixture of cellulase and pentosanase led to separation of germ and endosperm. Since cellulase alone effected no separation, the results suggest that the separation results from degradation of the pentosans of the cell walls.

Products of Enzymic Hydrolysis. Examination of the products obtained on hydrolysis of the prepared corn sections with fractions of Enzyme 19 confirmed the importance of the pentosans in separation of germ and endosperm. Cellulase yielded only a small amount of glucose and an unknown substance (R_G=0.33) after 24 hours of treatment. According to Whistler and Smart (11), cellobiose is the principal sugar formed during the first 3 hours of hydrolysis of cellulose with Enzyme 19AP. No cellobiose was found in the present study.

The pectic enzyme fraction yielded traces of glucose, xylose, arabinose, and possibly galactose after 24 hours of digestion. These sugars originated from the activity of the small amounts of pentosanase, cellulase, and amylase which contaminated the preparation. No galacturonic acid or other degradation products typical of pectic substances were found. These results are in agreement with those of the histochemical tests.

The fraction which contained cellulase and pentosanase yielded appreciable amounts of glucose, arabinose, and xylose after 2 hours of treatment. Galactose appeared later, together with a series of oligosaccharides. These decomposition products are typical of cell-wall hemicelluloses. Although quantitative data were not obtained, a steady increase in output of sugars was evident over the 24-hour period of treatment. Relatively large quantities of sugars were present after 5 hours in comparison with the traces formed by the other fractions of Enzyme 19 in 24 hours. The early appearance of arabinose and its relative abundance on the chromatograms suggest the probability that easily hydrolyzable arabinose side chains may be present in the hemicellulose.

Glucose, an appreciable part of which appeared early on the chromatograms, probably came from several sources. Some undoubtedly originated from degradation of the cellulose fraction, and some may have come from the small amount of starch retained within unopened endosperm cells after treatment with malt alpha-amylase. However, glucose from these two sources should appear at a relatively late stage.

The remainder of the glucose, particularly that which appeared at an early stage, probably came from degradation of the hemicellulose fraction of the cell walls by the pentosanase. Support for this view is provided by the fact that a small amount of glucose was found in a hydrolysate of starch-free germ cell walls obtained by treatment with hot 2% sulfuric acid solution. Such treatment should have little effect on the cellulose fraction of the cell walls.

Discussion

Microscopic examination of corn sections treated with enzyme fractions and identification of the products of enzymic hydrolysis showed clearly that cell-wall pentosans were readily susceptible to enzyme attack. The cellulose, on the other hand, was highly resistant to degradation by cellulase. Cellulase activity of the enzyme used on the soluble substrate, carboxymethylcellulose, was found to be relatively high. Only what has been designated by Reese *et al.* (8) as Cx activity is measured on this substrate. These authors have postulated that degradation of native cellulose involves two enzymes: The C1 system which splits native cellulose into linear chains of anhydroglucose, and the Cx system which splits the beta-1,4-glucosidic linkages in the linear chains to form small, soluble molecules. On the basis of this hypothesis, the low activity of the cellulase fraction from Enzyme 19 on native cellulose may be due to a deficiency of the C1 system. Whitaker (12), however, concluded from his work that a single enzymic system is involved in the hydrolysis of cellulose with the enzyme that he used. Degradation, in the present study, might have been slow because the native cellulose was not available to the enzyme.

On the basis of the high pentosan content alone, however, it is reasonable to expect pentosanase to cause extensive cell-wall degradation in the cereal grains. Mature corn pericarp, which consists mostly of cell-wall material, contains about 50% hemicellulose (15); wheat endosperm cell walls have from 25 to 50% hemicellulose (16). Preliminary work on corn germ cell walls indicates that the hemicellulose content is of the order of 50%.

Microscopic examination of enzyme-treated sections showed that the walls of the fingerlike epithelial cells of the scutellum are more easily degraded at the tips than near the base of the cells. The tip portions are also more easily degraded than any part of the cell walls of the scutellar parenchyma. This may result from a higher pentosan content in the walls at the tips of the epithelial cells, from a difference in the submicroscopic structure of this portion of the cell wall, or from both.

During germination of the kernel, the tips of the scutellar epithelial cells undergo a marked increase in size. The submicroscopic structure of the wall of this part of the cell must be relatively labile to permit expansion. Its greater susceptibility to enzymic attack may also depend on the submicroscopic structure. The tips of the epithelial cell walls, containing relatively soluble pentosans, are strategically located in the critical border area, immediately adjacent to the cementing layer. An attack upon them seems to offer more practical possibilities for efficient degermination than does an attack on the resistant cementing layer or on the compressed layer.

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EFFECT ON CRUMB FIRMNESS
I. Mono- and Diglycerides[1]

C. W. Offelt,[2] Majel M. MacMasters, Earl B. Lancaster, and F. R. Senti[3]

ABSTRACT

Crumb firmness of bread made with 20 formulas varying only in total fat and composition showed: Increase in the lard content from 1 to 4% caused no significant decrease in crumb firmness, which confirms statements in the literature. Monoglyceride decreased crumb firmness. This effect was apparently independent of fat level. Economical optimum effect of monoglyceride probably is about 0.25 to 0.3%. Whether obtained from lard or from cottonseed oil, diglycerides had no effect on crumb firmness and exerted no synergistic action to increase efficacy of monoglyceride when the two were used together.

Many changes have occurred during the past 20 years in methods of distributing bread from bakeries. Such changes have emphasized the relationship between age of bread and its acceptability by the ultimate consumer. One major change that takes place as bread ages is the firming of the crumb. Carlin *et al.*[4] reported that modified fats affect the rate of firming. Edelmann and Cathcart (1) included monoglyceride in their studies of the effectiveness of 24 different surface-active agents as emulsifiers or "softeners" in bread. In their studies, as in most others, the action of monoglycerides at arbitrary levels of 0.5 and 1.0% was reported. Establishment of the standards for identity of bread in 1952 (2) introduced the requirement that monoglyceride present be not over 10% of the total added fat and when used with diglycerides, not over 8% of the total fat because of added softening action of the diglycerides. This qualification implies specific synergistic action of the diglycerides.

The Northern Utilization Research and Development Division recently initiated a study of the relationship between chemical structure of compounds when used as additives in bread and their effect on crumb firmness. There was a possibility that information on softening action obtained with monoglycerides at the 0.5 and 1.0% levels might not be applicable at restricted levels. Some doubt has been expressed, moreover, as to the effect of diglycerides. The first part of the study was therefore confined to determination of the effect of mono-

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[4] Carlin, G. T., Hopper, R. P., and Thomas, M. J. Factors pertaining to the tenderness and staling of bread. Presented at 32nd annual meeting, AACC, Kansas City, Mo., May 1947.

and diglycerides on crumb firmness. The effect of monoglyceride at different levels was studied; and the effect, if any, of diglycerides when used alone, or in combination with monoglyceride, was investigated. The results obtained are summarized in this paper.

Materials and Methods

Materials. The fat used in the control and with the additives was commercial deodorized lard. The monoglyceride was a commercial distilled product (Myvatex 7-40)[5] incorporated with lard to give a mixture of 70% monoglyceride and 30% lard. Additions to formulas were made on the basis of actual monoglyceride content. Allowance for lard in the mixture was made in adding lard to the formulas in which the mixture was used. The diglycerides were also distilled products and included samples from both vegetable (cottonseed) and animal (lard) sources.

The flour used was a 2:1 blend of hard red winter wheat flour and hard red spring wheat flour. Both were commercial baker's patents, bleached, with approximately 12.0% protein and 0.42% ash.

Baking Procedure. A straight dough procedure was employed. Mixing was to optimum development, as observed by the operator, for each formula. A 2.5-hour total fermentation, at 86°F. (30°C.) and 85% relative humidity, was used with a "punch" at 105 minutes, scaling after 135 minutes, and panning at 150 minutes. The doughs were panned in standard 1-lb. tins and proofed at 95°F. (35°C.) and 85% relative humidity to 5/8 in. over the top of the pan. Baking was for 30 minutes at 425°F. (218.3°C.). Loaves were weighed and loaf volumes were determined by the seed displacement method immediately upon removal from the oven. The bread was cooled for 1 hour in a controlled atmosphere (78°F. (25.6°C.) and 40% relative humidity) and wrapped in a double waxed glassine paper for storage in cabinets at 78°F. (25.6°C.) and 40% relative humidity.

Firmness Determination. The firming rate was not determined because of limited facilities and personnel at the time the studies were made. Antifirming action was therefore determined by obtaining the differential in crumb firmness between the control and the test sample 45 hours after removal from the oven. This time was chosen for convenience; previous work had shown that increases in firmness after 24 hours are approximately linear in all cases.

A Modified Bloom Gelometer (see footnote 5) was used to measure crumb firmness, which was expressed as the grams of shot required to cause a depression of 4 mm. by a disk 1 in. in diameter. Three slices

[5] The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

at each end of the loaf were discarded, and firmness was determined on each of the remaining twelve slices. All slices were ½ in. thick.

Experimental Design

Laboratory equipment available mixed only enough dough for two loaves at a time. Previous experience had shown that the variability from mix to mix on the same day was about equal to that from day to day, and was considerably greater than that of loaves within a mix. An inverse relationship between loaf volume and firmness was evident for a variety of bread formulations. The experiments were therefore arranged in a randomized block design to provide a uniform environment for comparing the formulations: One mix, each, of ten formulas was prepared and baked in one day, and the program was repeated to provide five replications. Each replication included duplicate loaves.

Interpretation of Results. The data were subjected to an analysis of covariance and the mean firmness for each formula was adjusted to a common loaf volume for the design. The adjusted values were more meaningful, since any effect the additive may have on firmness through its effect on loaf volume is thus separated from its independent effect on firmness alone. The validity of this reasoning was proved in a separate experiment in which a different proofing method was used to obtain more nearly constant loaf volume.

Results and Conclusions

The effect of monoglyceride level on crumb firmness is demonstrated in Fig. 1. The length of the black and crosshatched bars indicates the range of values, including three tests at the 0 level of monoglyceride and two tests at each of the other levels except at 0.4% where only one was made. The white and crosshatched bars are the unadjusted values and are given for comparing the direction of change due to adjustment of the firmness to a loaf volume of 2770 cc. Within the monoglyceride levels are mixes representing total fat levels from 1 to 4%, with the restriction that the monoglyceride does not exceed 10% of the total fat. Although with no monoglyceride the increased amount of fat seemed to exhibit some antifirming properties, these experiments do not show the effect to be significant, and it disappears on addition of monoglyceride. Monoglyceride definitely decreased crumb firmness and it is evident that the effect of monoglyceride decreased markedly above about 0.25 to 0.3%. An optimum economical level for use of monoglyceride by the baker is thus indicated.

The data obtained from a second set of ten formulas are summarized in Table I. Two of the formulas contained 4% lard as shortening. The action of the monoglyceride in decreasing crumb firmness

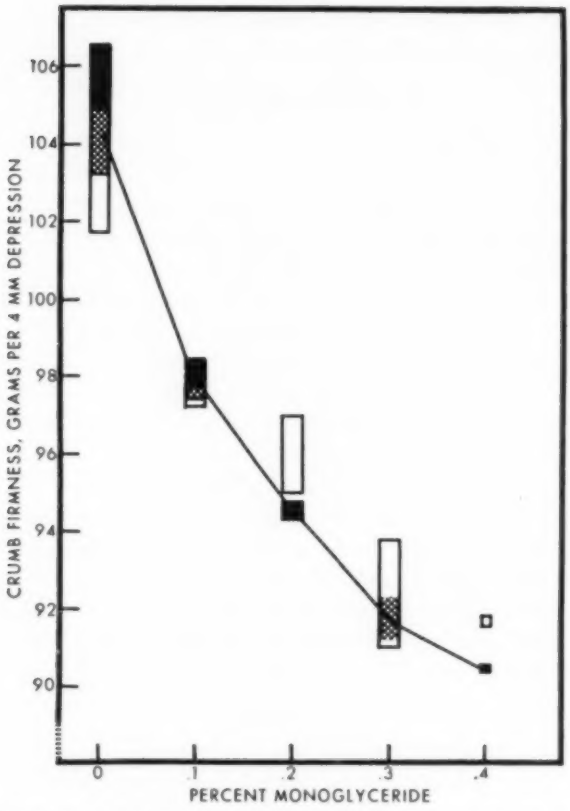

Fig. 1. Effect of monoglyceride level on crumb firmness. Dark areas indicate adjusted values; light areas, unadjusted values; barred areas, overlapping of unadjusted and adjusted values.

TABLE I

CRUMB FIRMNESS OF BAKES WITH LARD AND WITH ADDED MONO- AND DIGLYCERIDES, ADJUSTED MEANS[a]

SHORTENING COMPOSITION		ADJUSTED FIRMNESS VALUE
4% Lard (two formulas)	108.2 / 106.1 \ average	107.2
0.1% Monoglyceride + 3.9% lard		104.3
0.4% Monoglyceride + 3.6% lard		96.8
0.1% Lard diglyceride + 3.9% lard		108.2
0.4% Lard diglyceride + 3.6% lard		106.0
0.4% Monoglyceride + 0.6% diglyceride + 3.0% lard		95.6
0.66% Commercial mixture of 60% mono- and 40% diglyceride + 3.34% lard		95.1
0.1% Cottonseed diglyceride + 3.9% lard		107.7
0.4% Cottonseed diglyceride + 3.6% lard		107.9

[a] The firmness values have been adjusted to an average loaf volume of 2770 cc. Standard error of adjusted means is 1.2.

was confirmed. The diglycerides showed no effect on crumb firmness when added alone to replace part of the lard, and gave no evidence of synergistic effect when added with the monoglyceride. This was true whether the diglyceride came from cottonseed or from lard. The current assumption that diglycerides exert a synergistic antifirming action on crumb when added with monoglyceride appears to be completely erroneous.

Acknowledgments

The authors are grateful to the Distillation Products Co., Rochester, N. Y., for the monoglyceride and both diglycerides; to R. T. Vanderbilt Co., New York, N. Y., for the commercial mixture of mono- and diglycerides; to Carol B. Burnett, Henry L. Seckinger, and William E. Schulze for aid in the laboratory; and to Morris D. Finkner for aid in statistical interpretation of the data.

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EFFECT ON CRUMB FIRMNESS
II. Action of Additives in Relation to Their Chemical Structure[1]

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ABSTRACT

3-Stearoyl-D-glucose was as effective an antifirming agent for bread as polyoxyethylene monostearate. Ascorbyl palmitate, isoascorbyl palmitate, and stearoyl-D-glucose were all about as effective as monoglyceride. Ascorbyl palmitate and ascorbyl stearate imparted a brownish color to the crumb.

Chain length of the fatty acid portion of the molecule appeared to have some effect on the antifirming action of the additive, but it was evident that other structural factors must also be considered.

Antifirming agents are of acknowledged importance to the baking industry through their minimizing effect on stale "returns." Of the numerous factors involved in the aging of bread, firming is one of major importance because it is by the firmness of the loaf that the average housewife judges its freshness. Complete control of all other changes during aging would be practically useless if firming remained uninhibited.

The beneficial effect of monoglycerides as antifirming agents in bread has been established and these compounds are now in use. It has been shown that polyoxyethylene monostearate has an even greater antifirming action than monoglyceride. However, the use of this compound is banned.

It has been pointed out by Kass[4] that compounds which retard the firming of bread crumb are surface-active agents, and that the location and amount of electrostatic charge are important characteristics of such compounds. No systematic study has appeared in the literature to indicate specifically how structural differences in additive compounds affect their antifirming action. Such a study was undertaken, therefore, at the Northern Utilization Research and Development Division to determine the relationship between chemical structure and the antifirming effect of possible additives. The work reported here is one phase of this study.

Materials and Methods

The flour and the fat used in the controls and with the additives have been previously described (3).

[1] Manuscript received June 20, 1957. Presented at the 42nd annual meeting, San Francisco, May 1957.

[2] Present address: DCA Food Industries, Inc., 45 W. 36th St., New York 18, N. Y.

[3] Northern Utilization Research and Development Division, Peoria, Illinois, one of the Divisions of the Agricultural Research Service, U. S. Department of Agriculture.

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The 3-stearoyl-D-glucose and 3-palmitoyl-D-glucose were prepared by esterification of 1,2:5,6-diisopropylidene-D-glucose (1) with fatty acid chloride followed by acid hydrolysis for removal of the isopropylidene groups. Their structures should be considered as tentative, having been assigned on the basis of method of preparation and assuming no acyl migration during removal of isopropylidene groups. Confirmation of structure presently is in progress. Stearoyl-D-glucose and stearoyl- and palmitoyl-D-gluconolactones were prepared by action of the respective fatty acid chlorides on D-glucose and D-glucono-delta-lactone in pyridine solution. Acetylation of ascorbyl palmitate with acetic anhydride produced the triacetyl derivative, and catalytic hydrogenation yielded the reduced compound. Ascorbyl stearate was prepared by the method of Swern *et al.* (4). Synthesis of the N-substituted D-gluconamides (2) and of dimethylamino-hexose-reductone[5] have been previously described.

The methods employed in baking and in analyzing the data were the same as those described in a previous publication of this series (3).

Each replicate in Table I represents four mixes each of the addi-

TABLE I

EFFECT OF VARIOUS TYPES OF COMPOUNDS ON THE CRUMB FIRMNESS OF BREAD

ADDITIVE IN SHORTENING (0.4% Additive + 3.6% Lard)	NUMBER OF REPLICATE FORMULATIONS	DEVIATION OF ADJUSTED FIRMNESS VALUE FROM THAT OF CONTROL[a]
Polyoxyethylene monostearate	4	−18
Monoglyceride	4	−12
Tartaric acid ester of monoglyceride	2	− 8
Calcium stearyl-2-lactylate[b]	1 each	(0.25%) − 3
		(0.50%) − 4
3-Stearoyl-D-glucose	2	−18
3-Palmitoyl-D-glucose	2	− 2
Stearoyl-D-glucose	1	−12
Sucrose monostearate	1	− 6
Methyl alpha-D-glucoside stearate	1	− 1
Ethyl alpha-D-glucoside stearate	1	− 5
Ascorbyl stearate	3	− 7
Ascorbyl palmitate	6	−13
Ascorbyl myristate	2	− 7
Isoascorbyl palmitate	2	−10
Reduced ascorbyl palmitate	3	− 9
Triacetyl ascorbyl palmitate	1	− 8
Stearoyl-D-gluconolactone	1	− 6
Palmitoyl-D-gluconolactone	1	+ 1
N-octyl-D-gluconamide	1	+ 7
N-Dodecyl-D-gluconamide	1	+13
Dimethylamino-hexose reductone	1	− 7

[a] All values adjusted to a loaf volume of 2770 ml. Errors for comparing differences in antifirming effects range from 2 to 6.

[b] Instead of usual 0.4% additive, 0.25 and 0.50% were used.

[5] Hodge, J. E., Fisher, B. E., and Rist, C. E. Preparation and properties of amino-reductones from reaction of hexoses with amines. Abstracts, 125th meeting, Am. Chem. Soc., Kansas City, Mo., March 1954.

tive and the control run on a single day. The *maximum* error of the difference representing antifirming action is estimated as 2, expressed as standard error. The least significant difference (LSD) is then about 4 for the 5% level of significance. The precision, of course, improves with repeated runs.

Results and Discussion

The results are summarized in Table I. Neither the tartaric acid ester of monoglyceride nor calcium stearyl-2-lactylate proved to be as effective an antifirming agent as the monoglyceride which, in turn, was less effective than polyoxyethylene monostearate.

The most striking result of this study is the finding that 3-stearoyl-D-glucose is as effective an antifirming agent as polyoxyethylene monostearate. This discovery may have particular interest and significance because 3-stearoyl-D-glucose is derived from two naturally occurring, edible compounds – stearic acid and glucose.

A further advantage of 3-stearoyl-D-glucose is that doughs prepared with it are "drier" than untreated doughs, hence machinability is improved. Also, additional water may be introduced into the mix treated with 3-stearoyl-D-glucose to yield doughs equivalent in mobility to comparable doughs not treated with this antifirming agent.

Of all the products tested, only the glucose derivative substituted at carbon atom 3, the ascorbic and isoascorbic acid derivatives substituted at carbon atom 6, and the N-substituted gluconamides have unequivocal structures. Sucrose monostearate probably was predominantly substituted at carbon atom 6 of the glucose moiety (5). The other products, from the manner of synthesis, were expected to be of indefinite structure.

It would appear that in the case of substances of known structure, length of the carbon chain of the fatty acid residue is a critical factor in obtaining proper lipophilic-hydrophilic balance for good antifirming action. However, increase in softening action with fatty acid chain length could not be established for all of the classes of substances investigated.

Comparison of the results obtained shows that 3-stearoyl-D-glucose is a more effective antifirming agent than its homolog, 3-palmitoyl-D-glucose. Although N-dodecyl-D-gluconamide has bread-firming action, its lower fatty amine homolog N-octyl-D-gluconamide produces a lower firming effect. An irregular pattern of fatty acid effect is found with the ascorbic acid derivatives. For example, ascorbyl palmitate is more effective than either the myristate or the stearate. Considerable browning of the bread crumb is obtained with these derivatives, but less browning

occurs when reduced ascorbyl palmitate is used. However, triacetyl and reduced ascorbyl palmitates are not as effective as ascorbyl palmitate, whose antifirming effect is comparable to that of monoglycerides. The isomeric isoascorbyl palmitate behaves similarly. With products of irregular structure which were synthesized in analogous manner, a fatty acid chain length effect is also observed. Stearoyl-D-gluconolactone has better softening action on bread than the palmitoyl derivatives.

Position of the fatty acid residues in the carbohydrate structure may also be important for good softening action. The 3-stearoyl-D-glucose has a greater effect than D-glucose monostearate where the fatty acid is presumed to be irregularly substituted. It is of interest that the latter product is equivalent to monoglyceride in antifirming effect.

Methyl- and ethyl-alpha-D-glucoside stearates are approximately equivalent in action. Thus, the firmness value of stearoyl-D-glucose is not enhanced by glycoside formation. Dimethylamino-hexose-reductone has an enediol structure and in this respect resembles ascorbic acid. However, the reductone has no fatty acid substituent. Its antifirming effect is comparable to that of ascorbyl stearate.

Acknowledgments

The authors gratefully acknowledge the helpfulness of the companies which supplied the various commercial products used. The calcium-stearyl-2-lactylate was obtained from the C. J. Patterson Co., Kansas City, Mo.; the tartaric acid ester of monoglyceride from Hachmeister, Inc., Pittsburgh, Pa.; the polyoxyethylene monostearate from R. T. Vanderbilt Co., New York, N. Y.; the distilled monoglycerides from Distillation Products Co., Rochester, N.Y.; the methyl- and ethyl alpha-D-glucoside stearates from E. F. Drew & Co., Boonton, N. J.; sucrose monostearate from Foster Dee Snell, Inc., New York, N. Y.; and ascorbyl palmitate from the Eastern Utilization Research and Development Division, Wyndmoor, Pa. Thanks are also given to J. E. Hodge and E. C. Nelson for the preparation of reduced ascorbyl palmitate; to W. E. Schulze, H. L. Seckinger, and J. E. Hubbard for their aid in the baking laboratory; and to E. B. Lancaster and M. D. Finkner for advice in statistical interpretation of data.

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STUDIES ON CORN PROTEINS

II. Electrophoretic Analysis of Germ and Endosperm Extracts[1]

EDWIN T. MERTZ,[2] NORMAN E. LLOYD,[3] AND RICARDO BRESSANI[4]

ABSTRACT

Electrophoretic patterns of an alkaline copper extract and of a sodium chloride extract of the corn germ from a sample of U. S. corn were similar in conformation. Fractionation of alkaline copper extracts of the endosperm from a U. S. and a Guatemalan sample of corn gave three fractions, an acid-soluble fraction, an alkali-, alcohol-soluble fraction (zein), and an alkali-soluble, alcohol-insoluble fraction (glutelin). These three fractions accounted respectively for 23.4, 46.9, and 24.3% of the endosperm nitrogen in the U. S. sample, and 24.3, 44.7, and 21.0% of the endosperm nitrogen in the Guatemalan sample. Each of the electrophoretic components of the alkaline copper extracts of the endosperm from the U.S. and Guatemalan corn samples could be identified as an electrophoretic component of one or more of the three fractions. Electrophoretic analysis of a saline extract of U. S. endosperm revealed four major components having mobilities similar or identical to four components of the alkaline copper extract. With the exception of a starch component, each of the electrophoretic components of the acid-soluble fractions of the U. S. and Guatemalan endosperms had a mobility similar or identical to one of the components of the zein or glutelin fraction. This suggests that either albumins and globulins partially coprecipitate with the alkali-soluble, acid-insoluble proteins, or that only proteins of the zein and glutelin types are found in corn endosperm.

It was reported previously (5) that the proteins of corn germ and endosperm can be rapidly and almost completely extracted by an alkaline medium containing sodium, copper, sulfate, and sulfite ions. A method for the separation of the endosperm extracts into three fractions is described below. These fractions, the whole copper extracts of germ and endosperm, and saline extracts of germ and endosperm have been subjected to electrophoretic analysis.

Materials and Methods

Two corn samples were studied: U. S. 13 precursor, a single-cross hybrid yellow dent corn containing 1.68% nitrogen, and Tiguisate (TGY), a yellow open-pollinated flint-type corn from the Guatemalan lowlands, containing 1.57% nitrogen. Endosperm and germ were separated by manual dissection as described previously (5). The U. S. endosperm contained 1.56% nitrogen and represented 82.3% of the dry weight of the kernel; the germ contained 3.1% nitrogen and repre-

[1] Manuscript received March 11, 1957. Journal Paper No. 1084, Purdue University Agricultural Experiment Station, Lafayette, Indiana. The data in this paper are taken from a thesis submitted by N. E. Lloyd in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biochemistry.

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sented 11.3% of the dry weight. The Guatemalan endosperm contained 1.5% nitrogen and represented 81.3% of the dry weight of the kernel, whereas the germ contained 2.7% nitrogen and represented 12.25% of the dry weight. Ten-gram samples of ground, defatted (5) endosperm, and 2-g. samples of ground, defatted germ were used for the copper extraction-fractionation studies.

The extraction of ground, defatted germ and endosperm with copper reagent followed the published method (5), except that the pH of the extraction was 11.5 instead of 12. The copper extracts of germ were not fractionated further (see ref. 2) but were used in this form for electrophoresis studies. The copper extracts of the endosperm were fractionated as described below.

Fractionation of U. S. and Guatemalan Endosperm Extracts. The residue insoluble in copper reagent is designated as fraction I in the fractionation diagram (Fig. 1). The extract plus "washing" (5) is labeled "combined supernatants" in Fig. 1. In order to obtain a more complete removal of copper ions, this solution was adjusted to pH 3.0. A 200-ml. aliquot was then centrifuged at 2,000 r.p.m. for 10 minutes.

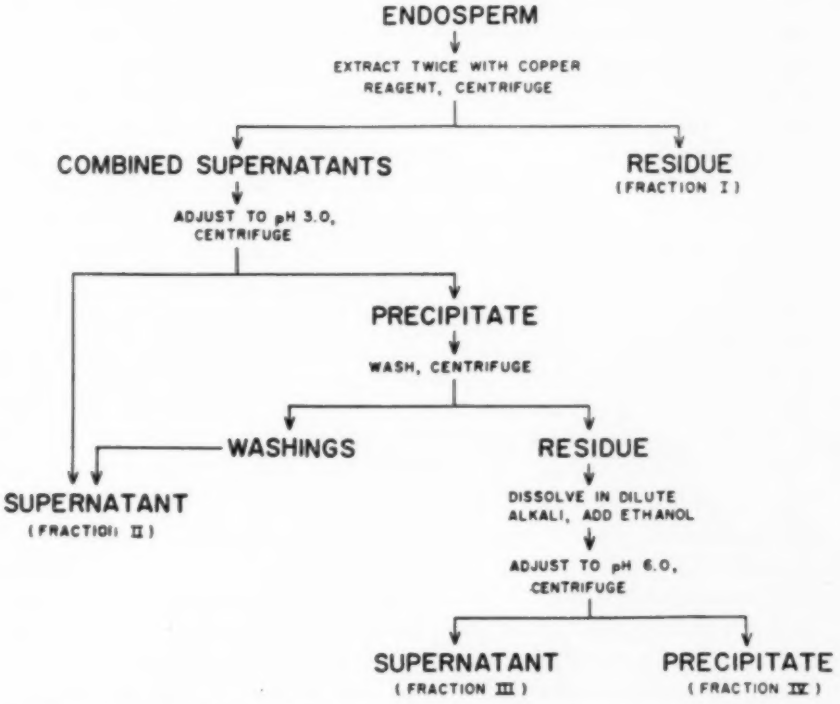

Fig. 1. Diagram of method used to separate corn endosperm into four fractions.

the supernatant saved, and the precipitate washed in a beaker by stirring for 20 minutes, first with 50 ml. of pH 3.0 hydrochloric acid solution, and then with 50 ml. of water. The washings were removed by centrifugation and combined with the original supernatant, this solution constituting the acid-soluble fraction (fraction II, Fig. 1). The residue was saved. This fractionation was carried out at room temperature.

To the washed residue (insoluble at pH 3.0), were added 15 ml. of water and just enough dilute (pH 11.5) sodium hydroxide solution to dissolve the residue. The volume of solution was then brought to 30 ml. with water, and 60 ml. of absolute ethanol were added. The clear solution was adjusted to pH 6.0 with 10% acetic acid, and placed in a refrigerator overnight. The glutelins precipitated from solution, and were removed by centrifuging at 2,000 r.p.m. for 30 minutes (fraction IV, Fig. 1). The supernatant alcohol solution contained the zein (fraction III, Fig. 1).

Saline Extract of Endosperm. A sample of ground defatted U. S. endosperm (18 g.) was extracted with 200 ml. of 5% sodium chloride solution buffered at pH 6.8 with 0.1*M* sodium phosphate buffer. The mixture was stirred for 24 hours at 0 to 2°C. The slurry was centrifuged at 2,000 r.p.m. for 1 hour, and the supernatant decanted and stored at 0°C. The residue from this extraction was re-extracted three times in the same manner. The supernatants from all extractions were combined and filtered through a fine sintered glass filter. The saline extract was dialyzed against deionized water and lyophilized.

Saline Extract of Germ. Two grams of ground, defatted U. S. germ were suspended in 10 ml. of pH 8.2 borate buffer (0.1 molar), and frozen and thawed four times. Forty milliliters of 12.5% sodium chloride solution were added to the thawed sample, and the mixture was stirred rapidly for 3 hours at room temperature. After centrifugation at 2,000 r.p.m. for 1 hour, the supernatant was decanted and saved, and the residue extracted 2 hours with 50 ml. of buffered salt solution (5 parts borate buffer, 20 parts 12.5% sodium chloride solution). This was repeated with 20 ml. of buffered salt solution and an extraction time of 1 hour. The supernatants from all of the extractions were combined. The saline extract was dialyzed against deionized water and lyophilized.

Preparation of Extracts for Electrophoretic Analysis. Endosperm: The whole endosperm extracts (combined supernatants, Fig. 1) and fraction II (Fig. 1) were dialyzed against pH 3.0 hydrochloric acid solution until the dialysates no longer gave a test for copper ion. Fraction III (Fig. 1) was dialyzed against pH 11.3 sodium glycinate buffer

to remove the ethanol. All were then dialyzed exhaustively against deionized water to remove ions, and lyophilized. Fraction IV (Fig. 1), obtained as a wet precipitate, was washed several times with water and lyophilized.

Germ: The whole copper extract of U. S. germ was dialyzed against pH 3.0 hydrochloric acid solution, followed with deionized water as described above for endosperm extract, and lyophilized.

Electrophoretic Analyses. All analyses were carried out on the lyophilized products in a Perkin-Elmer Model 38 electrophoresis apparatus at 0°C. using a 6-ml. cell. The products were completely soluble in the sodium veronal or sodium glycinate-sodium chloride (3) buffers used (see legends, Figs. 2 and 3). The patterns obtained were enlarged and traced on graph paper. Electrophoretic mobilities were calculated from the patterns made of the descending boundaries.

Nitrogen Determinations. Duplicate aliquots of the extracts of germ and endosperm, and of the supernatants obtained in the fractionation steps, were analyzed by the micro-Kjeldahl method (1). The data were used to compute percentage distribution of nitrogen among the various fractions. The nitrogen lost by dialysis of the whole copper extract of endosperm, and the aqueous solution of fraction II, was estimated as follows. Aliquots of these solutions, containing known amounts of nitrogen, were placed in 4-in. lengths of viscose tubing, and the solutions dialyzed against frequent changes of pH 3.0 hydrochloric acid solution to remove copper ions. The viscose tubes plus contents were analyzed for total nitrogen, and the values corrected for the nitrogen content of empty 4-in. lengths of viscose tubing soaked the same length of time in pH 3.0 hydrochloric acid solution.

Results

Nitrogen Distribution in Endosperm. Extraction of the U. S. endosperm with the copper reagent yielded 94.6% of the total endosperm nitrogen. Fraction I (residue) therefore contained 5.4% of the total endosperm nitrogen. On further fractionation (see Fig. 1) the percentage distribution of the total endosperm nitrogen was as follows: fraction II, 23.4%; fraction III, 46.9%; and fraction IV, 24.3%.

In contrast, extraction of the Guatemalan endosperm with copper reagent yielded 90.0% of the total endosperm nitrogen. On further fractionation, the percentage distribution of the total endosperm nitrogen was as follows: (fraction I, 10%) fraction II, 24.3%; fraction III, 44.7%; and fraction IV, 21.0%. The degree of reproducibility of this fractionation is indicated by the values obtained by a different analyst (2) using another portion of the same sample of Guatemalan Tiguisate.

The nitrogen distribution in the endosperm was: fraction I, 8.6%; fraction II, 23.4%; fraction III, 45.7%; and fraction IV, 22.3%.

In the studies reported above, 10-g. samples of ground, defatted endosperm were extracted and fractionated. It is of interest to note that fractionations permitting calculation of nitrogen distribution have been made recently[5] with 0.6-g. samples of ground, defatted endosperm.

Treatment of the U. S. endosperm with 5% buffered sodium chloride solution extracted 17.3% of the total nitrogen. Of the 17.3%, 5.4% was dialyzable, leaving 11.9% as "protein" nitrogen. Similarly, 10.7 of the 94.6% of the total nitrogen in the whole copper extract of U. S. endosperm was dialyzable, leaving 83.9% as "protein" nitrogen. Most of the dialyzable nitrogen of the whole copper extract is found in fraction II (Fig. 1), where it makes up 8.1 of the 23.4%, leaving 15.3% as "protein" nitrogen.

It is of interest to compare our results with those of Osborne and Mendel (6). When they extracted their sample of corn endosperm with 10% potassium chloride solution, 7.8% of the total nitrogen was recovered in the extract. Using 5% sodium chloride solution, we extracted 17.3% of the nitrogen from our corn endosperm sample. One-half of their total nitrogen was "90% ethyl alcohol-soluble," compared with 46.9% accounted for in our zein fraction (fraction III). Their "0.2% potassium hydroxide-soluble" fraction accounted for 38% of the total nitrogen, whereas our glutelin fraction (fraction IV) accounted for 24.3% of the total nitrogen.

It is probable that our corn sample has a lower glutelin content than the sample of Osborne and Mendel, for about 10% more of the total nitrogen is soluble in saline solution. Thus, it appears that the copper extraction-fractionation method yields zein and glutelin fractions which are quite similar to those obtained by the classic Osborne-Mendel method. However, the possibility that the copper method may cause more severe alterations in the native corn proteins should not be overlooked; this has been discussed at some length in the first paper of the series (5).

Nitrogen Distribution in the Germ. With 10% sodium chloride solution, 82% of the U. S. germ nitrogen was extracted; this is in fairly good agreement with the 77.2% of germ nitrogen extracted by Osborne and Mendel with 10% potassium chloride (6). A total of 91% of the nitrogen in the germ of our U. S. corn sample was extracted with the copper reagent. The extract was not fractionated further; however, in later work (2) the germs and endosperms of several corn varieties were fractionated according to the scheme outlined in Fig. 1 and, in con-

[5] Mertz, E. T., and Mourkides, G. Unpublished data.

trast to the endosperm, the bulk of the nitrogen of the germ was about equally divided between the acid-soluble and alkali-soluble fractions of the copper extract (Fig. 1, fractions II and IV).

Electrophoretic Analysis of Endosperm Fractions. Electrophoretic patterns of the total protein extracted from U. S. endosperm with the copper reagent, and of its fractions II, III, and IV, are shown in Fig. 2.

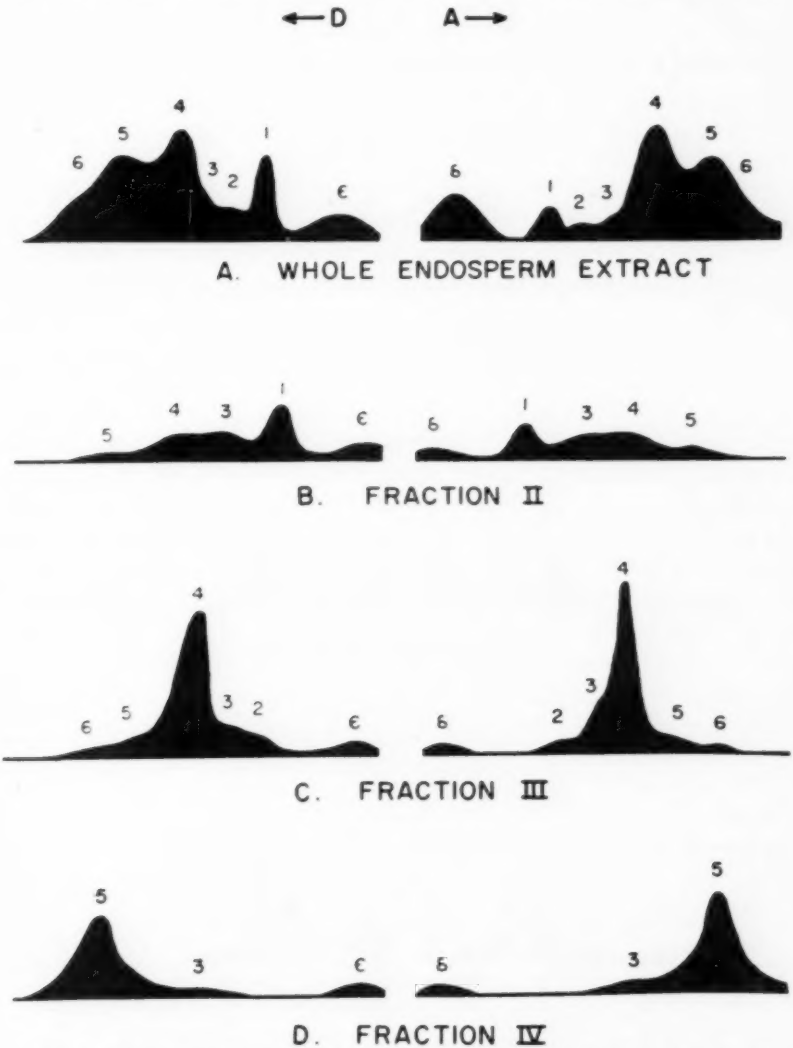

Fig. 2. Electrophoretic patterns of endosperm fractions. Protein, A to C respectively: 0.79, 0.153, 0.45, and 0.33%. All preparations run in pH 11.6 sodium glycinate-sodium chloride buffer, ionic strength 0.1, for 180 minutes at 4.5 volts/cm.

All of the protein in the endosperm extract is recovered in these three fractions. The pattern of the proteins in the 5% sodium chloride extract of U. S. endosperm is shown in Fig. 3, A. Patterns for the Guatemalan endosperm proteins are not shown. All of the protein fractions were inhomogeneous. A total of six electrophoretic components can be identified in the whole U. S. endosperm extract (Fig. 2, A), which range in mobility from -1.9×10^{-5} cm^2 volt^{-1} sec^{-1} for component 1, to -6.5×10^{-5} cm^2 volt^{-1} sec^{-1} for component 6. With the exception of fraction IV, the mobilities of the components numbered alike (Fig. 2, B, C, D) show good agreement (Table I). This was con-

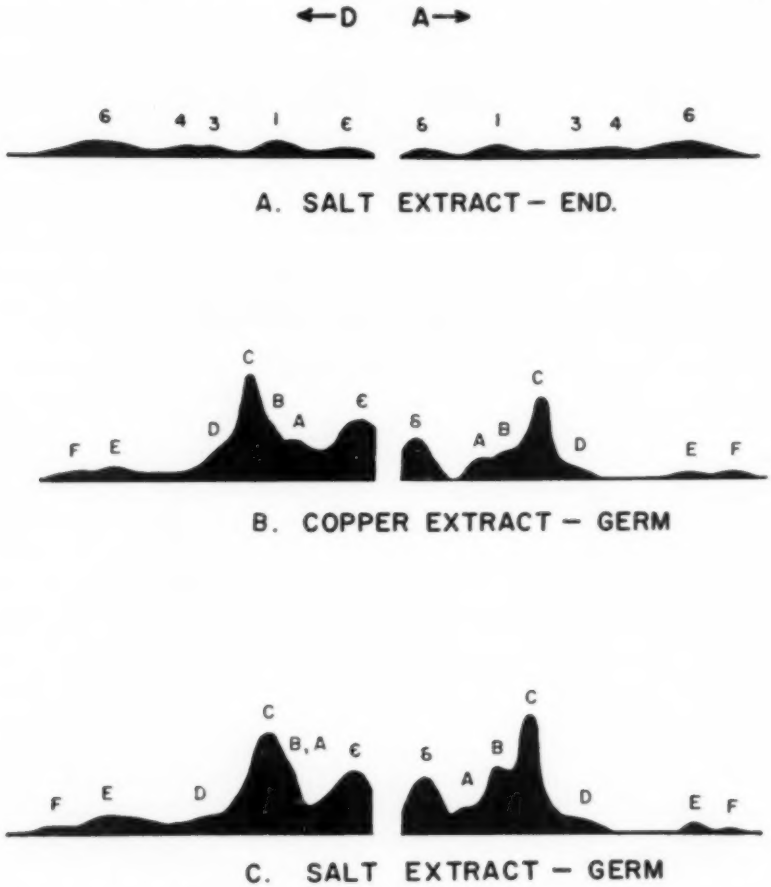

Fig. 3. Electrophoretic patterns of germ and endosperm fractions. A, 0.12% protein, run in pH 11.6 sodium glycinate-sodium chloride buffer, ionic strength 0.1, for 180 minutes at 4.5 volts/cm. B, 0.91% protein, and C, 0.82% protein, run in pH 8.6 sodium veronal buffer, ionic strength 0.1, for 80 minutes at 5.6 volts/cm.

TABLE I

ELECTROPHORETIC MOBILITIES OF COMPONENTS FROM U.S. ENDOSPERM

ELECTROPHORETIC COMPONENT	WHOLE ENDOSPERM EXTRACT[a]	FRACTION II[b]	FRACTION III[c]	FRACTION IV[d]	SALT EXTRACT[c]	MOBILITY RANGE
1	1.9[f]	2.0	..	..	1.9	(1.9–2.0)
2	2.7	..	2.6	..	..	(2.6–2.7)
3	3.6	3.6	3.2	4.0[g]	3.5	(3.2–3.6)
4	4.0	4.3	4.0	..	4.3	(4.0–4.3)
5	5.5	5.5	5.6	6.6[g]	..	(5.5–5.6)
6	6.5	..	6.5	..	6.3	(6.3–6.5)

[a–d] Respectively, parts A, B, C, and D, Fig. 2.
[e] Part A, Fig. 3.
[f] Multiply by -1×10^{-5} cm^2 volt^{-1} sec^{-1}. All mobilities at pH 11.6.
[g] An error in timing gave these high values in the electrophoretic analysis of fraction IV shown in Fig. 2, part D. A more accurate analysis of fraction IV is shown in Table II. See text.

firmed with Tiguisate endosperm (Table II), which in addition showed good agreement between the components of fraction IV and the same components of the whole endosperm extract.

Comparison of the data in Tables I and II shows good agreement between the mobilities of the individual components of similar fractions, when one considers the differences in corn varieties and the slightly higher pH used for analysis of the U. S. endosperm fractions. As pointed out in a footnote to Table I, the mobilities of the components of fraction IV (D in Fig. 2) are too high. In support of this conclusion and in confirmation of the data obtained on fraction IV from Tiguisate endosperm (Table II), four separate electrophoretic runs on samples of fraction IV from the U. S. endosperm, which were made later in glutelin studies, gave mobilities of -5.6 to -5.7×10^{-5} cm^2 volt^{-1} sec^{-1} for the major component.

It is of interest to note that component 4 was not found in the patterns of fraction II in the Guatemalan endosperm (Table II). In contrast to U. S. endosperm (Table I), this component was apparently at such a low level that it could not be resolved in the presence of components 3 and 5, which flank it on both sides.

TABLE II

ELECTROPHORETIC MOBILITIES OF COMPONENTS FROM GUATEMALAN ENDOSPERM

ELECTROPHORETIC COMPONENT NO.	WHOLE ENDOSPERM EXTRACT[a]	FRACTION II[b]	FRACTION III[a]	FRACTION IV[b]	MOBILITY RANGE
1	1.6[c]	1.6	..	..	.....
2	2.6		2.2	..	(2.2–2.6)
3	3.4	3.3	2.8	3.4	(2.8–3.4)
4	4.4		4.2	..	(4.2–4.4)
5	5.1	5.3	5.5	5.5	(5.1–5.5)
6	6.0	..	6.2	..	(6.0–6.2)

[a] Mobilities at pH 11.5.
[b] Mobilities at pH 11.3.
[c] Multiply by -1×10^{-5} cm^2 volt^{-1} sec^{-1}.

Inspection of the electrophoretic data shows that of the components present in the total protein extracted from endosperm, component 1 is recovered only in fraction II (acid-soluble), most of component 4 is recovered in fraction III (zein), and most of component 5 is recovered in fraction IV (glutelin). Inspection of the pattern and mobilities of the proteins extracted with salt solution (Fig. 3, A; Table I) reveals that components are present (components 1, 3, 4, and 6) whose mobilities agree closely with those contained in fractions II, III, and IV (Figs. 2, B, C, D; Table I). The components of fraction II (except for component 1) are found to be similar electrophoretically to components of the alcohol- and alkali-soluble fractions (Tables I and II).

All of the preparations submitted to electrophoretic analysis were tested for the presence of starch by acidifying a small portion and adding iodine-potassium iodide reagent. The salt extract, the whole endosperm extracts (copper method), and fraction II gave positive tests, whereas fractions III and IV did not. When the nitrogen content of fraction II (U. S. endosperm) was compared with the nitrogen content predicted from its electrophoretic pattern by the area method, the results did not agree unless the area under peak 1 (component 1) was excluded from the calculation. It is therefore quite probable that component 1 is starch and not protein. In earlier studies, extraction of endosperm with 0.1*M* sodium sulfite at pH 2.0 yielded a major electrophoretic component which was not amphoteric. Upon separation by electrophoresis, it gave a very positive iodine test and contained only 0.5% nitrogen. We believe that component 1 (fraction II) is this polysaccharide.

Soluble Proteins of Endosperm Fractions. The electrophoretic data obtained on saline extract, whole copper extract, and copper extract fractions of endosperm show that the endosperm contains two major protein fractions, an alcohol- and alkali-soluble fraction (our fraction III or "zein") and an alcohol-insoluble, alkali-soluble fraction (our fraction IV or "glutelin").

The mobility data (Tables I and II) suggest that nonzein nonglutelin protein components of the salt- and acid-soluble fractions of endosperm are related to components 3, 4, 5, and 6 of fraction III (zein) and to components 3 and 5 of fraction IV (glutelin). There are several possible explanations of their similarity to components of fractions III and IV. These proteins may be albumins and globulins that partially coprecipitate with the acid-insoluble proteins. However, it is more likely that they are polymers similar to proteins in the zein and glutelin fractions, but with a lower order of polymerization, or

other properties permitting greater solubility in aqueous neutral and acidic media.

Support for the latter hypothesis is found in the fact that the major component of the glutelin fraction (component 5) has a soluble counterpart in fraction II of both U. S. and Guatemalan endosperm. In addition, the major component of the zein fraction (component 4) has a soluble counterpart in both the salt extract and fraction II of U. S. endosperm.

Electrophoretic Analysis of Germ Extracts. At least six components ranging in mobility from -2.2 to -14.6×10^{-5} cm^2 volt^{-1} sec^{-1} were present in the extracts of germ. The similarity in conformation between the electrophoretic patterns of the germ proteins extracted with alkaline copper reagent (B, Fig. 3) and with 10% sodium chloride solution (C, Fig. 3) suggests that the proteins extracted with copper reagent are similar to those extracted with sodium chloride solution. Mobilities calculated from the ascending patterns were as follows (components A to F, respectively): Copper extract, 2.9, 4.2, 5.7, 7.4, 12.2, and $14.3 \times -1 \times 10^{-5}$ cm^2 volt^{-1} sec^{-1}. Saline extract, 2.2, 3.5, 5.0, 7.6, 12.6, and $14.6 \times -1 \times 10^{-5}$ cm^2 volt^{-1} sec^{-1}. Electrophoretic analyses of the copper extract fractions of germ (2) are needed for further identification of components A to F (Part B, Fig. 3).

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STUDIES ON CORN PROTEINS
III. The Glutelins of Corn[1]

NORMAN E. LLOYD[2] AND EDWIN T. MERTZ[3]

ABSTRACT

Buffered solutions of whole glutelin prepared from an alkaline copper extract of corn were separated by differential centrifugation into three fractions, a coarse fraction (one-third of the protein), an alpha-plus-beta fraction, and a gamma fraction. On the basis of sedimentation data it is estimated that the alpha-fraction proteins had molecular weights in excess of 1 million, the beta-fraction proteins 100,000 to 200,000, and the gamma fraction proteins 10,000 to 20,000. Both the whole glutelin and the gamma-fraction proteins had the same mobility and migrated under a single peak in the Tiselius electrophoresis cell. The whole glutelin and gamma-fraction proteins contained more aspartic acid, arginine, cystine, glycine, lysine, tryptophan, and valine, and less glutamic acid, isoleucine, leucine, proline, and serine than zein. Physical measurements showed that the gamma-fraction proteins were highly elongated molecules.

Much is known about the properties of zein, the alcohol-soluble fraction of corn endosperm proteins. Its amino acid composition, solubility properties, film- and fiber-forming tendencies, and nutritive value have been studied extensively (11). In comparison, relatively little is known about the properties of glutelin, the alkali-soluble, alcohol-insoluble fraction of the endosperm.

Whole glutelin was prepared from ground defatted corn by a modification of a copper extraction and fractionation method described previously (6). The physical properties of the whole glutelin and one of its fractions were investigated by electrophoresis and by preparative and analytical ultracentrifugation; in addition, a quantitative method was used to determine the amino acid composition of the whole glutelin and one of its fractions.

Materials and Methods

Preparation of Corn Sample. A sample of a yellow single-cross hybrid corn was used in the following experiments. The corn was first ground in a burr mill at a medium setting, then at the finest setting, to give a particle size of 0.001 in. Powdered dry ice was mixed with the corn before it was ground the second time, to prevent an excessive temperature rise. The ground corn was extracted with hexane for 48 hours in a Soxhlet apparatus and then air-dried overnight.

[1] Manuscript received March 11, 1957. Journal Paper No. 1085, Purdue University Agricultural Experiment Station, Lafayette, Indiana. The data in this paper are taken from a thesis submitted by N. E. Lloyd in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biochemistry.

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Extraction of Proteins. The ground, defatted corn was extracted with an alkaline reagent containing copper and sulfite by a slight modification of the procedure described by Mertz and Bressani (5). The ground, defatted corn was suspended in fifteen parts of a water solution containing seven parts of copper sulfate pentahydrate and one part of sodium sulfite for every 100 parts of dry corn sample, and the pH of the mixture raised to 11.5 by adding 1 molar sodium hydroxide solution with stirring. After the addition of the sodium hydroxide, the slurry was stirred for 1 to 2 hours at room temperature,

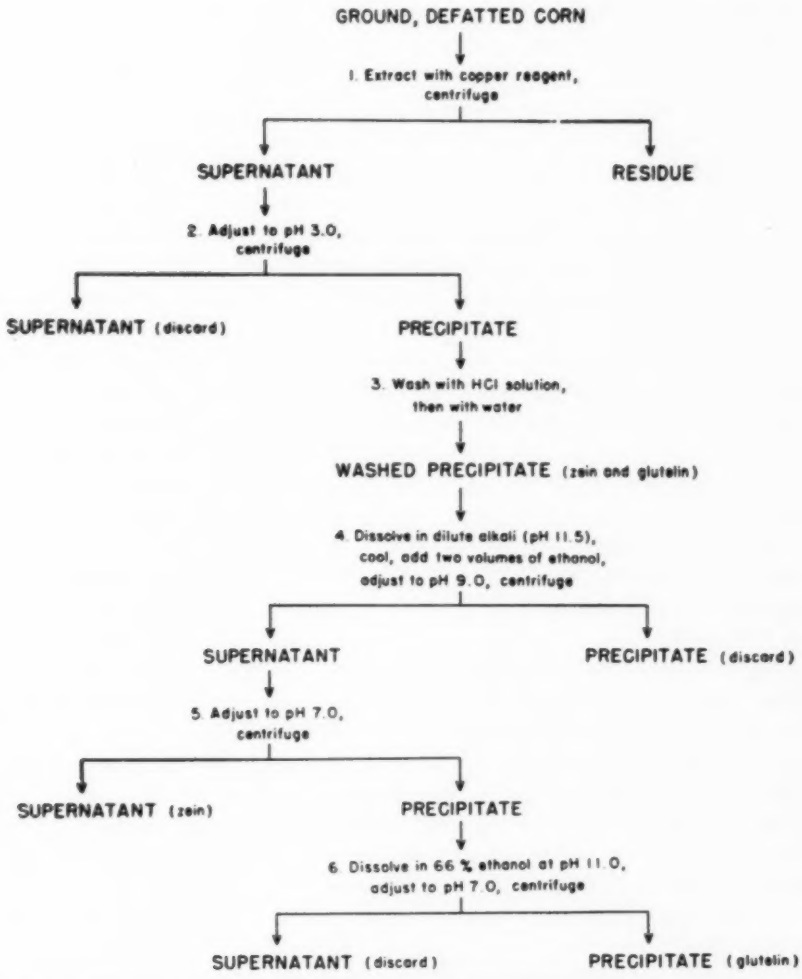

Fig. 1. Diagram of steps used in the preparation of glutelin from whole corn.

and was then centrifuged at 2000 r.p.m. for 10 minutes. The supernatant was decanted and used in the fractionation procedure described below.

Preparation of Whole Glutelin. Figure 1 shows the steps followed for the preparation of whole glutelin. All of the steps (4, 5, and 6), wherein the proteins were in contact with alcohol, were carried out at −5 to −8°C. by means of a refrigerated bath. Centrifugations were carried out at −5°C. in a refrigerated centrifuge (International PR1). All pH values were measured with a glass electrode standardized at 25°C. with pH 10.00 buffer.

The supernatant obtained in step 1 from the extraction of ground corn with the copper reagent was adjusted to pH 3.0 with 4*N* hydrochloric acid. The precipitate which formed was collected by centrifuging at 2000 r.p.m. for 15 minutes.

The wet precipitate from step No. 2 was suspended in 5 to 10 times its weight of hydrochloric acid solution (pH 3.0) and was washed with this solution (to remove residual copper) by stirring for 30 minutes. The suspension was centrifuged at 1000 r.p.m. for 3 minutes, the supernatant decanted, and the precipitate rewashed by the same procedure three times with pH 3.0 hydrochloric acid solution, and three times with water.

The washed precipitate from step 3, which was a mixture composed primarily of zein and glutelin, was dissolved in dilute sodium hydroxide solution at pH 11.5 to a concentration of 1 to 2% protein. The solution was placed in a flask and cooled to 0°C. in a refrigerated bath. Two volumes of 95% ethanol for every one volume of alkaline protein solution were added dropwise over a period of 4 to 8 hours while the solution was cooling to −8 to −5°C. At this point, a precipitate of protein and retrograded starch formed.[4] The mixture was adjusted to pH 9.0 with 10% acetic acid and centrifuged for 1 hour at 2000 r.p.m.

The supernatant obtained from step 4 was transferred to a beaker and the pH adjusted to 7.0. A precipitate of glutelin formed which was collected by centrifuging for 10 minutes at 2000 r.p.m.

The precipitate of glutelin from step 5 was dissolved in alkaline, aqueous ethanol (two parts by volume of 95% ethanol to one part water) at pH 11.0 to a final concentration of approximately 1% protein. The pH of the alkaline solution was adjusted to 7.0 with 4*N* hydrochloric acid to reprecipitate the glutelin.

The reprecipitated glutelin from step 6 was collected by centrifuga-

[4] In the previous paper (6), a precipitate was not obtained at this point in the fractionation of corn endosperms. The difference is due to the use of whole corn instead of endosperm, and a temperature of −5°C. instead of room temperature. Thus, the germ protein not soluble in step 2 is removed in steps 4, 5, and 6 (Fig. 1).

tion and traces of alcohol and salts washed from it with several small portions of cold water. It was then lyophilized and stored over phosphorus pentoxide.

Isolation of the Gamma-Glutelin Component. One to two per cent solutions of lyophilized whole glutelin were made up in pH 10.5 sodium glycinate-sodium chloride buffer, ionic strength 0.1, as follows. The whole glutelin was suspended in water, and 1*N* sodium hydroxide added with stirring to pH 11.5, to dissolve the glutelin. The solutions were then dialyzed against a large volume of pH 10.5 buffer for 24 hours at 0°C.

The dialyzed solutions were centrifuged at 40,000 r.p.m. for 13 hours at 0 to 4°C. (Spinco, Model L, Head No. 40). The rotor was allowed to decelerate slowly without benefit of the electronic brake to minimize stirback and convection of the tube contents. The top four-fifths of the supernatants were carefully removed from each centrifuge tube and were pooled. The supernatants obtained were perfectly clear, but slightly orange-colored.

The pooled supernatants were then dialyzed against several changes of deionized water and were lyophilized and stored at −20°C. Yields of the gamma component by this method of preparation were 15 to 25% of the original whole glutelin used as starting material.

Measurement of Physical Constants. Electrophoretic analyses were carried out in a Perkin-Elmer Tiselius apparatus at 0°C. using a cell of 2-ml. capacity. Electrophoretic mobilities were calculated from patterns made of the descending boundaries.

Sedimentation analyses were carried out in a Spinco Model E Ultracentrifuge. Preparation of the whole glutelin sample for analysis was as follows. Three hundred milligrams of whole glutelin were dissolved in 30 ml. of sodium glycinate-sodium chloride buffer at pH 11.2. The resulting solution was clarified by centrifuging at 30,000 r.p.m. (Spinco, Model L, Head No. 40) for 30 minutes. Aliquots of the supernatant were analyzed by the micro-Kjeldahl method (1). One-half of the supernatant was dialyzed against pH 10.5 sodium glycinate-sodium chloride buffer, and the other half dialyzed against pH 8.6 sodium veronal buffer.[5] Parallel analyses of these two solutions were carried out using a rotor equipped with a standard and a prismatic cell. Individual analyses of *gamma*-glutelin solutions were carried out using a rotor equipped only with the standard cell.[6]

Diffusion experiments on gamma-glutelin were carried out in a

[5] All buffers were 0.1M in sodium ion and 0.1 ionic strength. The proportions of glycinate, chloride, and veronal ion were adjusted to give the required pH.

[6] We are indebted to Dr. B. Roger Ray, Department of Chemistry, University of Illinois, for the double-cell analyses, and to Dr. J. F. Foster, Department of Chemistry, Purdue University, for providing a Spinco Model E and instructions to one of us (N.E.L.) for the standard-cell analyses.

Perkin-Elmer Tiselius apparatus employing a modified Longsworth optical system for recording schlieren diagrams. Gamma-glutelin was dissolved in buffer and the solution dialyzed against the buffer overnight. An electrophoresis cell of 2-ml. capacity was filled with the solution to be analyzed as for a normal electrophoresis run, except that the electrodes were not used. The temperature of the cell was maintained at 0 ± 0.1°C. by means of an ice bath. The boundary was moved to the center of the cell immediately after its formation (by alignment of the electrophoresis cell parts) by adding buffer to one side of the cell assembly through a tube attached to a motor-driven compensator (4). Schlieren diagrams were then recorded on film at eight or ten intervals over a period of 40 hours. The film recordings were placed in an enlarger and the enlarged images traced on graph paper.

Diffusion coefficients were calculated from the enlarged tracings by the maximum ordinate-area method (4). The areas under the diffusion curves were calculated with a mechanical integrator.[7] Axial ratios (a/b) were calculated from the corrected sedimentation and diffusion constants, assuming that the molecules approached the shapes of prolate ellipsoids of revolution and that the degree of hydration was 0.5 g. of water per g. of protein.

Amino Acid Analyses. Approximately 20-mg. samples of whole glutelin and of gamma-glutelin were weighed out in glass tubes. Five-milliliter aliquots of 6*N* hydrochloric acid were added to the tubes and the tubes sealed and placed in an oven for 22 hours at 110 ± 2°C. They were removed and allowed to cool to room temperature. The cooled tubes were then opened and placed in a desiccator at reduced pressure (5 cm. Hg) over a mixture of solid sodium hydroxide pellets and anhydrous calcium chloride, in order to evaporate the acid solution. The residues of the hydrolysates after evaporation were dissolved in 5-ml. portions of pH 3.39 sodium citrate buffer. The amino acid analyses of the whole glutelin and gamma-glutelin hydrolysates were made in duplicate, and the results averaged. Corrections for partial destruction of serine, threonine, and tyrosine were made using the values published by Moore and Stein (9).

One-milliliter aliquots of the buffered hydrolysate solutions were chromatographed on columns of Dowex 50 as described by Moore and Stein (7). The 1-ml. effluent fractions from the columns were analyzed by a variation of the colorimetric method of Cocking and Yemm (3). Ninhydrin reagent was prepared according to the directions of these authors, with the exception that 4.0*M*, pH 5.51 acetate buffer was substituted for the 0.2*M* citrate buffer so that adjustment of the pH

[7] Keuffel and Esser, Compensating Polar Planimeter, 4236.

of the effluent fractions from the columns of Dowex 50 could be avoided (8). The 1-ml. effluent fractions were analyzed in batches of 40, freshly prepared ninhydrin reagent being used for each batch. Color values for each of the common amino acids were determined as described by Moore and Stein (8). Standard solutions of all of the amino acids listed in Table II were chromatographed in order to check the resolution and recovery of each amino acid from the columns. Recovery of the amino acids averaged $100 \pm 2\%$. The separation of proline from glutamic acid and of cystine from glycine and alanine was incomplete, in contrast to the results of Moore and Stein, so that these amino acids were determined by other methods.

Cystine (plus cysteine if present) was converted to cysteic acid and determined as described by Schramm, Moore, and Bigwood (12). The cysteic acid content of the hydrolysates of the performic acid-treated proteins was determined by chromatography on a 100-cm. column of Dowex 50 as described by Moore and Stein (7). The values found for cysteic acid were then converted to units of cystine.

Proline was determined by the colorimetric method of Troll and Lindsley (14). Aliquots of the same acid hydrolysates of whole glutelin and gamma-glutelin as were used for chromatography were used for these analyses. Absorbance values of the solutions after color development with the acidic ninhydrin reagent were determined in a Coleman Junior Spectrophotometer and the absorbance values converted to units of proline by reference to a standard curve.

Tryptophan was determined on unhydrolyzed samples of whole glutelin and glutelin in duplicate by the colorimetric method of Spies and Chambers (13).

Amide nitrogen was determined by the method of Pucher, Vickery, and Leavenworth (10). Fifteen-milligram samples of the proteins were heated at 100°C. for 4 hours in 1*N* sulfuric acid. The amide ammonia released by this hydrolysis procedure was determined as described by these authors. A Beckman Spectrophotometer (Model DU) was used to determine the absorbances of the Nesslerized ammonia solutions.

Other Analyses. Samples of whole glutelin and gamma-glutelin were dried in a vacuum oven (1 cm. Hg) for 1 hour at 110°C. The nitrogen contents of the dried products were then determined by the micro-Kjeldahl method (1).

Tests were made for the presence of carbohydrate in the protein preparations by using a 10% solution of alpha-napthol in ethanol, and concentrated sulfuric acid (Molisch test). Other tests for carbohydrate were made, one with a 1% solution of orcinol (5-methyl-1,3-benzenediol) in 100% sulfuric acid, and another with a 0.4% solution

of anthrone (9,10-dihydro-9-ketoanthracene) in concentrated sulfuric acid. For the latter tests, 5 mg. of the protein preparations were suspended in 1 ml. of water and 3 ml. of the sulfuric acid solution of orcinol or anthrone added and mixed well. A positive test with the orcinol reagent was a red color and with the anthrone reagent, a blue-green color. Tests with these reagents on crystalline pepsin, bovine serum albumin, water, and a mixture of corn proteins and starch were run as controls.

Results

The whole ground corn kernel, rather than ground endosperm, was used as the starting material for the preparation of glutelin, thus eliminating the time-consuming manual dissection of corn. Contamination of the glutelin fraction by proteins of the germ was not serious,

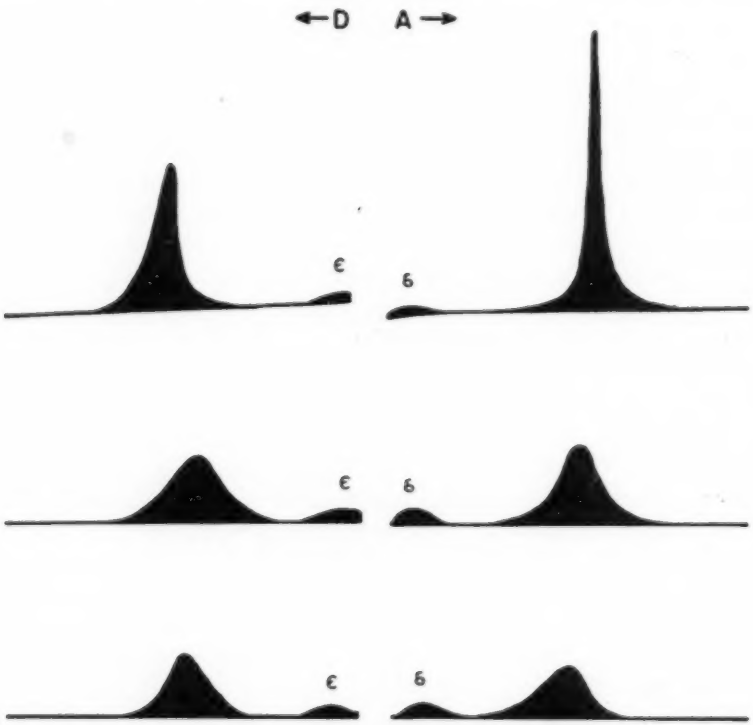

Fig. 2. Electrophoretic analysis of glutelins. *Top row:* Analysis of whole glutelin in pH 11.2 sodium glycinate-sodium chloride buffer, ionic strength 0.1, for 97 minutes at 5.8 volts/cm. *Center row:* Analysis of gamma-glutelin in pH 10.5 sodium glycinate-sodium chloride buffer, ionic strength 0.1, 112 minutes at 4.9 volts/cm. *Bottom row:* Analysis of gamma-glutelin in pH 8.6 sodium veronal buffer, ionic strength 0.1, 98 minutes at 5.6 volts/cm.

inasmuch as the germ proteins were removed in step 2 of the fractionation scheme (Fig. 1), and in subsequent steps. This is shown by the electrophoretic analysis of glutelin prepared from whole corn. The glutelin migrated as a single component in pH 11.2 glycine buffer with a mobility of -5.7×10^{-5} cm^2 volt^{-1} sec^{-1} (Fig. 2, top row). This mobility is in agreement with that of the major component of fraction IV of corn endosperm (6).

Approximately 20 per cent of the total nitrogen of the corn was recovered in the glutelin fraction isolated by the method outlined. The lyophilized whole glutelin was a light gray powder containing 16.1% nitrogen (dry weight basis), and could be dissolved in pH 11.5 buffer to give a slightly cloudy, yellow solution. The lyophilized product gave weak, but positive, tests for carbohydrate with the Molisch, the orcinol, and the anthrone reagents, indicating that carbohydrate material was present.

Sedimentation and Diffusion Analyses. The sedimentation analyses of the clarified glutelin in pH 10.5 glycine buffer (upper image) and in pH 8.6 veronal buffer (lower image) are shown in Fig. 3. Clarification

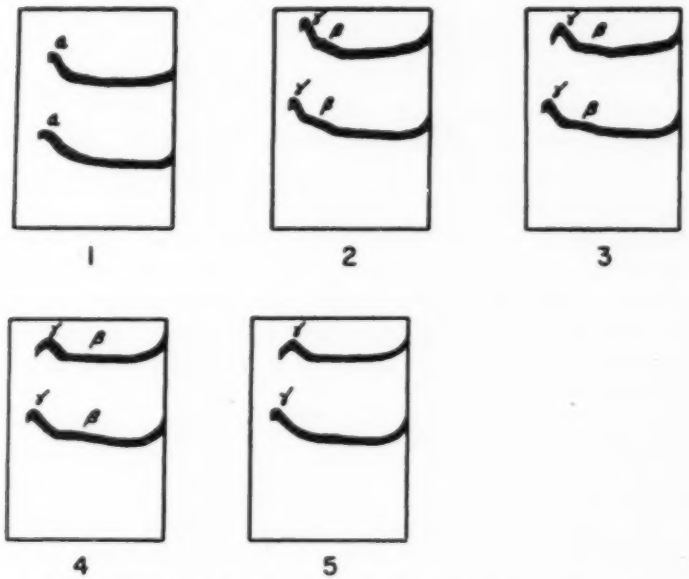

Fig. 3. Sedimentation analysis of clarified glutelin solutions. Upper image is of 0.65% glutelin solution in pH 10.5 sodium glycinate-sodium chloride buffer of ionic strength 0.1. Lower image is of 0.65% glutelin solution in pH 8.6 sodium veronal buffer of ionic strength 0.1. First exposure taken at 42,000 r.p.m. 2 minutes after starting acceleration of the rotor. Subsequent exposures taken at 8-minute intervals at 59,700 r.p.m. Direction of sedimentation is to the right.

of the solutions by centrifugation at 30,000 r.p.m. prior to analysis (see previous section) caused 35% of the protein of each preparation to be sedimented, so that the sedimentation analyses shown here represent the remaining 65% of the protein.

The analysis in the pH 10.5 buffer shows that three components are present. The first exposure, taken at a speed of 42,000 r.p.m. 2 minutes after the start of the acceleration of the ultracentrifuge rotor, shows the formation of a peak due to relatively gross material, which sedimented completely before the second exposure was taken. This was the fraction of highest molecular weight, which possessed a wide distribution of molecular sizes. The second exposure (10 minutes after acceleration was started) shows the presence of two more components, one a rather small component, and another larger and more slowly sedimenting component. The small component spread rapidly as it sedimented, indicating a rather wide distribution of sizes.

For the sake of discussion, the various components shown by the sedimentation diagram have been named the alpha-, beta-, and gamma-glutelin components in the order of decreasing sedimentation rate. The sedimentation coefficient (S_w, 20) of the gamma-component was 1.6 Svedberg units and that of the beta-component, 8.0 Svedberg units. This indicates that the estimated possible molecular-weight range of the beta-component in the pH 10.5 glycine buffer is 100,000 to 200,000, and that of the gamma-component is 10,000 to 20,000. No sedimentation coefficient for the alpha component was obtained, but it is estimated that this component possesses an average molecular weight of greater than 1 million.

The analysis of clarified glutelin in the pH veronal buffer paralleled the analysis in the pH 10.5 glycine buffer except that the rate of sedimentation of the gamma-component was considerably less in the veronal than in the glycine buffer. This difference in sedimentation was investigated as described later, using preparations of the purified gamma-component.

Several attempts were made to redissolve the protein which had sedimented during the centrifugation procedure for the isolation of the gamma-glutelin component from clarified or whole glutelin. However, the sedimented protein, consisting mostly of the alpha- and beta-glutelin components, had formed a tough, rubberlike plug in the bottoms of the centrifuge tubes and could not be redispersed by stirring in pH 11.5 glycine buffer or in copper reagent at pH 12.0 for 2 to 3 weeks. It should be pointed out, however, that both the alpha- and beta-glutelin components were completely "soluble" and well dispersed prior to ultracentrifugation at 40,000 r.p.m.

The lyophilized, gamma-glutelin obtained by the method described was a white powder containing 16.2% nitrogen. It could be dissolved readily in pH 8.6 veronal or in pH 10.5 glycine buffer to form clear, slightly orange-colored solutions. The preparation dispersed slowly in veronal buffer at pH 6.1 to form cloudy solutions, and was insoluble in buffers of pH 1.9, 2.5, and 4.4. The preparation of gamma-glutelin gave positive qualitative tests for carbohydrate material with the three testing reagents used.

Sedimentation analyses of the gamma-component are shown in Fig. 4. The gamma glutelin used in this case was prepared directly from the whole glutelin of corn several months after the preparation of the clarified glutelin used in Fig. 3. The top row shows the sedimentation at pH 10.5 and the bottom row the sedimentation at pH 8.6. The rate at which the boundaries spread as the component sedimented indicates that the component is not entirely monodisperse but that a rather symmetrical distribution of particle sizes exists. The sedimentation constants (S_w, 20) of the component at pH 10.5 and 8.6, the diffusion constant (D_w, 20) measured at a concentration of 0.5% protein, and the molecular weight and axial ratio of the gamma-component are shown in Table I. The data suggest that the molecular weight is dependent to a slight extent on the pH of the medium, being somewhat less at pH 8.6 than at 10.5. The observed difference in

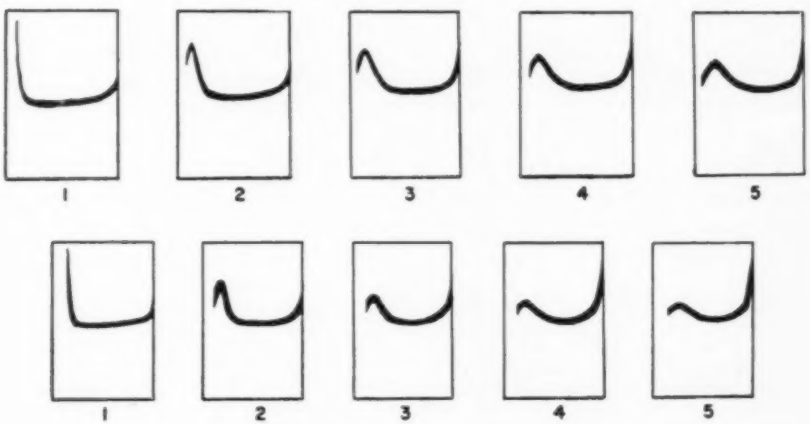

Fig. 4. Ultracentrifugal analysis of gamma-glutelin. Upper row of exposures is of 0.6% gamma-glutelin in pH 10.5 sodium glycinate-sodium chloride buffer, ionic strength 0.1. First exposure 13 minutes after a rotor speed of 59,780 r.p.m. was attained, with subsequent exposures at 16-minute intervals. Average temperature was 2.8°C. Lower row of exposures is of 0.4% gamma-glutelin in pH 8.6 sodium veronal buffer, ionic strength 0.1. First exposure 2 minutes after a speed of 59,780 r.p.m. was attained. Subsequent exposures, 32-minute intervals. Average temperature of run, 9.4°C. Direction of sedimentation is to the right.

TABLE I

PHYSICAL CONSTANTS OF GAMMA-GLUTELIN

CONDITIONS	SEDIMENTATION CONSTANT $\times 10^{13}$ (S_w, 20)	DIFFUSION CONSTANT $\times 10^{7}$ (D_w, 20)	MOLECULAR WEIGHT[a] (M_{SD})	AXIAL RATIO[b] (a/b)
	second	*cm² sec⁻¹*		
pH 10.5	1.6	5.4	25,800	19
pH 8.6	1.3	5.4	21,000	22

[a] Calculated assuming a partial specific volume of 0.72 cc/g.
[b] Calculated assuming the molecule approaches the shape of a prolate ellipsoid of revolution.

molecular weight of the component is probably significant, since the sedimentation rate at pH 8.6 was noted to be lower than at pH 10.5 on two occasions. No satisfactory explanation can be given for this difference. The axial ratio calculated from sedimentation and diffusion (Table I) shows that the component is made up of highly elongated molecules, in this respect resembling zein (4).

Electrophoretic Analysis. The gamma-glutelin component migrated electrophoretically as a single component at both pH 10.5 and 8.6 as shown in Fig. 2. As in the sedimentation diagrams, considerable spreading of the boundaries is evident in the electrophoretic patterns, indicating slight electrical inhomogeneity. The electrophoretic mobilites of the gamma-component at pH 10.5 and 8.6 were -5.8 and -5.7×10^{-5}

TABLE II

AMINO ACID CONTENTS OF GLUTELIN AND GAMMA-GLUTELIN

AMINO ACID	GLUTELIN	GAMMA-GLUTELIN	ZEIN[a]
	%	%	%
Nitrogen	16.1	16.2	...
Alanine	9.77[b]	6.57[b]	11.4[c]
Arginine	5.51	5.97	1.8
Aspartic acid	7.33	8.16	5.6
Cystine	3.14	4.20	1.0
Glycine	5.06	5.79	0.0
Glutamic acid	18.46	18.46	26.6
Histidine	2.11	3.12	1.7
Isoleucine	4.14	3.27	7.3
Leucine	11.87	9.31	23.7
Lysine	3.60	3.96	0.0
Methionine	2.24	2.36	2.3
Phenylalanine	7.21	3.56	6.4
Proline	7.62	7.85	10.4
Serine	4.36	5.00	7.7
Threonine	3.72	4.50	3.0
Tryptophan	0.92	1.00	0.1
Tyrosine	4.94	5.70	5.2
Valine	6.18	5.96	3.0
Ammonia (amide)	1.47	1.67	...

[a] Block and Bolling, ref. 2.
[b] Grams per 100 g. of protein.
[c] Expressed on a basis of 16 g. of nitrogen (2).

cm^2 $volt^{-1}$ sec^{-1} respectively. These values are essentially identical with the value obtained with whole glutelin at pH 11.2 (Fig. 2).

Amino Acid Analyses. Table II shows the amino acid and amide contents of a sample of whole glutelin and a sample of the gamma-glutelin component. The amino acid content of zein (Block and Bolling, 2) is also given for comparison.

By the methods of analysis used, 94% of the dry weight of the whole glutelin and 92% of the dry weight of the gamma glutelin component could be accounted for as peptide-bound amino acids and amide ammonia. Ninety-five per cent of the total nitrogen of both preparations was accounted for. This is a satisfactory recovery, for part of the weight of the glutelin, and of the gamma-component, is made up of carbohydrate material, both preparations giving slight, but positive, tests for carbohydrate.

The data in Table II show that whole glutelin and gamma-glutelin are quite unlike zein in amino acid composition. Zein is devoid of glycine and lysine, and very low in tryptophan, whereas the glutelins contain about 5% glycine, 4% lysine, and 1% tryptophan. In addition, the glutelins contain more aspartic acid, arginine, cystine, and valine and less glutamic acid, isoleucine, leucine, proline, and serine than zein. One may conclude from these analyses that glutelin proteins have a higher biological value than zein proteins, assuming equal digestibility.

When a comparison is made between the amino acid contents of gamma-glutelin and whole glutelin, it is found that the levels of arginine, glycine, glutamic acid, lysine, methionine, proline, tryptophan, and valine are quite similar. These findings, together with the electrophoretic data, suggest a close chemical relationship between gamma-glutelin and the other components of whole glutelin. It is even possible that gamma-glutelin may serve as a repeating unit in the glutelin proteins of higher molecular weight which are represented by our alpha-, beta-, and coarse glutelin fractions.

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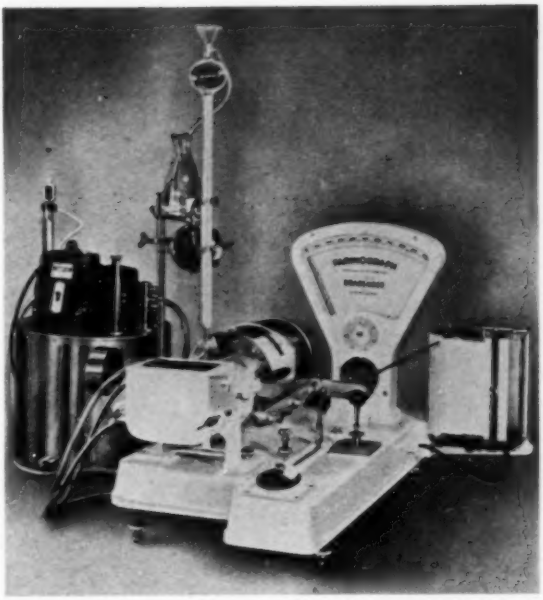

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